Modularity and Integration in the Skull of *Canis lupus* (Linnaeus 1758): A Geometric Morphometrics Study on Domestic Dogs and Wolves

DISSERTATION

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CONTENTS

1 INTRODUCTION

This doctoral thesis will focus on the cranium, the mandible, the teeth and the temporomandibular joints (TMJ) as subunits of the mammalian masticatory apparatus and decipher their integration and modularity in domestic dogs and wolves. Using *Canis lupus* (Linnaeus 1758) as a model species, the main objective of this thesis is to understand the developmental and structural dependence or independence of these skull parts, which either constrains or promotes the potential of the skull to evolve into new shapes not only in dogs and wolves but probably in the course of mammalian evolution in general.

Domestic animals show a morphological variation which surpasses the variation found in their ancestral form (Drake and Klingenberg, 2010; Young et al., 2017). This variation pertains almost all aspects of their morphology, from traits like fur, skin or feather colour and texture, body shape, size and posture (which includes skeletal modifications) and even behavioural traits (Trut, 2004; Herre and Röhrs, 2013; McGreevy et al., 2013; Wilkins et al., 2014; Young et al., 2017). Often the diversity reaches levels found only interspecifically or even higher taxonomic levels (Drake and Klingenberg, 2010; Young et al., 2017). One domestic animal in which the diversity of skull shapes is particularly striking is the domestic dog (*Canis lupus f. familiaris*, Linnaeus 1758) (Drake and Klingenberg, 2010). Here, the process of domestication of the grey wolf (Agnarsson et al., 2010) and artificial selection of the dog by humans resulted in over 350 different breeds now recognized by the Fédération Cynologique Internationale (FCI; www.fci.be), each with uniquely formed heads and skulls. This variation spans from brachycephalic, which describes a rostro-caudally short and medio-laterally wide skull, to dolichocephalic, which means a rostro-caudally long and medio-laterally narrow skull. Moreover, dog skulls vary from airorhynch, with a dorsally flexed rostrum, to klinorhynch, with ventrally flexed rostrum; and there are forms with either very pronounced or shallow sloping foreheads (Rosenberg, 1966; Nussbaumer, 1982; Brehm et al., 1985; Drake and Klingenberg, 2010; Drake, 2011). Not least, dog skulls vary enormously in size, which is associated with varying robustness or gracility of the skull (Klatt, 1949). Robustness here refers to a bulky appearance with accessory muscle attachment sites such as occipital and sagittal crests, wide zygomatic arches and a voluminous rostrum, while gracility means globular braincases without accessory muscle attachment sites, with narrow zygomatic arches and a slender rostrum (Klatt, 1949).

In the past, different approaches have been applied to explain the morphological variation of the domestic dog, which developed over the short evolutionary time span of probably 10’000 to 40’000 years (Skoglund et al., 2011; Botigué et al., 2017) and to understand the plasticity of the skull in spite of the limited genetic variation (Wayne and vonHoldt, 2012; Freedman et al., 2016). Most likely, this remarkable diversity is the combined outcome of various factors. Some of these are the natural variation among the founder populations of wolves, the repeated admixture with wolves throughout domestication history (Jolicoeur, 1959; Vila, 1999; Freedman et al., 2016) or specific genetic mutations which are thought to influence rostrum length and the development based on neural crest cells (Fondon and Garner, 2004; Cruz et al., 2008; Bannasch et al., 2010; Wayne and vonHoldt, 2012; Schoenebeck and Ostrander, 2013; Wilkins et al., 2014). Besides that, hormonal induction (Trut, 2004; Carrasco et al., 2014), degree and timing of cranial suture closure (Geiger and Haussman, 2016) as well as differing allometric constraints on the brain, the masticatory muscles and ultimately the skull (Klatt, 1949) have been suggested as influential factors. Other authors have argued for the retention of paedomorphic traits in domestic dogs (Wayne, 1986; Morey, 1992; Trut, 2004), a view that has been challenged by Drake (2011). Evidently, also age adds to the diversity of skull shapes (Rosenberg, 1966). Finally, the selective breeding for desired traits such as cute or bulky appearance (Fondon and Garner, 2004; Drake and Klingenberg, 2008), which was especially reinforced with the introduction of standardized breeding in Victorian times (Freedman et al., 2016), has contributed to the skull shape variation in modern dogs.

In the domestic dog, human husbandry has largely alleviated natural selective pressures on the functionality of the masticatory apparatus and the skull, which most likely enabled the radiation of skull shapes in the first place. Relaxed selective pressures and later strong artificial selection enabled even skull shapes which under natural conditions would dramatically reduce evolutionary fitness, such as the skulls of brachycephalic bulldogs or pugs with airway obstructions (Meola, 2013; Caccamo et al., 2014), malocclusion or disproportionally large teeth and undershot bites (McKeown, 1975; Colyer, 1990), or dachshunds with unusually shaped TMJs (Vollmerhaus and Roos, 1996). Similar disproportions and morphological anomalies also occur in wild wolves although they are reported less frequently and seem to occur in less extreme forms (Barrette, 1986; Vilà et al., 1993; Clutton-Brock et al., 1994; Federoff and Nowak, 1998). Thus, it is likely that natural selection for functionality effectively constrains variation in the masticatory apparatus and skull of the wolf, although the potential to rapidly develop similar disproportions still persists. This potential appears to be unleashed more frequently when

either selective pressures are lessened (e.g. in zoological gardens) or the gene pool is otherwise dramatically reduced (e.g. in small wild populations) (Federoff and Nowak, 1998; O'Regan and Kitchener, 2005). Similarly, in a domestication experiment, strong artificial selection had immediate effects on the skulls of farm foxes selected for tameness (Trut, 2004). Orofacial disproportions like the ones described raise the question in how far the TMJ, the teeth, and the anterior and posterior cranium and mandible are morphologically, genetically, and developmentally dissociated, and whether artificial selection altered the strength and pattern of their integration.

Integration in this context describes the degree to which two or more structures of the skull are structurally and developmentally connected (Klingenberg, 2008). Four levels may lead to integration: a shared development or genetic basis, a shared function or a shared evolution (Cheverud, 1996). Accordingly, parts of the skull in *Canis lupus* will be higher integrated if they 1) interact during development (e.g. originate from the same cell condensation), 2) are controlled by the same genes and are inherited together, 3) need to function in a coordinated fashion or are related to the same function, or 4) are integrated by evolution since selective pressures act equally on both parts (Cheverud, 1996). Integration in these categories can be realized by a diverse set of mechanisms in the skull: from forces acting throughout the skull which induce growth or reduction, over the distribution of signalling molecules to the genetic regulation of development and its inheritance (Esteve-Altava, 2017). Alterations in these mechanisms will lead to morphological changes which affect wide parts of the skull or even the skull as a whole.

As a counterpart to integration, there is modularity (Klingenberg, 2008). Modules are parts of the skull, which are integrated stronger internally than externally, to other parts of the skull (Eble, 2005; Klingenberg, 2008, 2009, 2013). Modularity thus is a dissociation, or “quasi-independence” (Wagner et al., 2007), of morphological traits which is caused by factors that are restricted to individual modules, such as genes affecting specific parts of a biological structure, cell condensations acting independently of others according to specific signals or local interactions with surrounding tissues (Klingenberg, 2008). In line with these factors (similar as for integration) different “types of modularity” have been defined in the past, from developmental modularity, to functional modularity, evolutionary or genetic modularity (Klingenberg, 2008). Yet, modularity and integration are no “either/or“ relationships. Rather both occur in most organisms in the one or the other way and on different hierarchical levels (Esteve-Altava, 2017) – from genes to whole body parts – and their balance can even change in the course of a lifetime (Polanski, 2011). In that way, skull variations can arise through both, dissociating or integrating factors at the

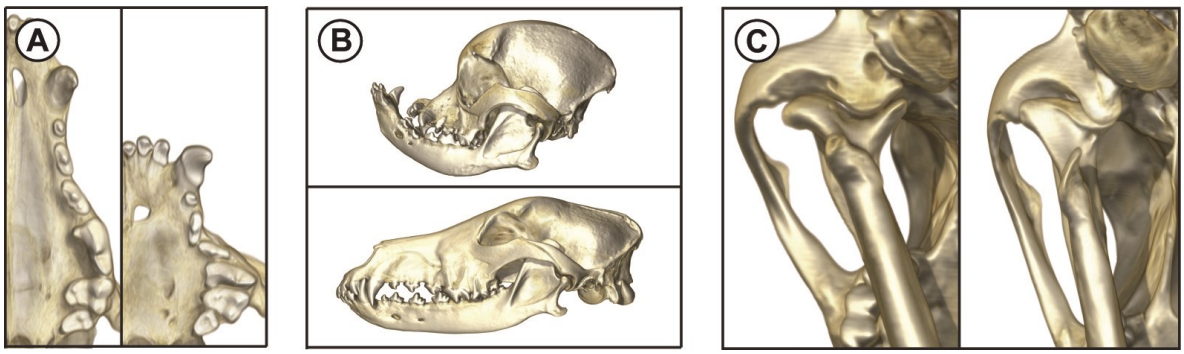

Fig. 1 Morphological phenomena which imply the reduced integration of the cranium, the mandible, the teeth and the TMJs in several dog breeds. A) crowded dentition in an English Bulldog (right) vs. uncrowded dentition of a German Shepherd (left), palatal view B) upper and lower jaw mismatch in an English Bulldog (top) and harmonious condition in a Collie (bottom), lateral view C) wavelike shape of the condylar process in a Dachshund (left), average jaw joint in a Collie (right), ventral view. The pictures are not to scale.

same time, allowing either variation which is restricted to single modules or variation which concerns the whole skull and possibly even the whole body. Usually, in a sample of adult specimens, it is not known which factors exactly lead to the integration or modularity of the structure studied (Esteve-Altava, 2017), which is why Mitteroecker and Bookstein (2008) subsumed factors that reinforce integration under the term “global factors” and factors that reinforce the dissociation of morphological traits as “local factors” without any further differentiation. Also in this thesis, the factors leading to the integration of the skull will be handled as a “black box”, since neither functional nor developmental or genetic factors have been tested here. The main objective of this thesis is to test whether structures are rather integrated or independent from each other, and whether the integration differs between domestic dogs and wolves regardless of underlying factors.

Aside from the balance of integration and modularity in mammalian skulls it is still under debate, how both relate to evolvability, which means the potential of a species to “respond” to selection (Klingenberg, 2008), and to the disparity of biological forms evolutionary processes can produce (Goswami and Polly, 2010; Sanger et al., 2012; Parr et al., 2016). On the one side, it has been advocated that a low integration of modules leads to increased diversity in the superordinate structure because single modules may vary more freely when variations have no negative impact on others. Following this line of argumentation the reduced integration of modules in the skull will increase their evolvability and thus facilitate the formation of new shapes in certain skull parts since it allows for more flexible solutions (Goswami, 2007; Zelditch et al., 2008; Kuratani, 2009; Marroig et al., 2009; Porto et al., 2009; Porto et al., 2013; Villmoare, 2013). Disproportions in the skulls of domestic dogs might be an expression of the potential of single modules to evolve (Fig. 1). On the other side, integration might have a positive impact on survival as

long as the selection pressure is along the trajectory of integrated variation (Villmoare, 2013). Thus, it has been argued, that most of the morphological variation might arise along “genetic lines of least resistance” (Schluter, 1996) meaning by altering “global factors” such as pleiotropic genes throughout the skull (Mitteroecker and Bookstein, 2008). Using the model of *Canis lupus,* this thesis will investigate how artificial selection has altered the balance of modularity and integration in domestic dog skulls when compared to the wolf as the non-domestic from, providing a possible explanation for the disparity of forms in domestic dog skulls.

In **Chapter 2.1**, the first study presented will test the hypothesis that artificial selection reduced the integration strength of the skull and altered the integration patterns among skull modules, allowing for greater disparity of skull shapes and for orofacial disproportions in domestic dogs. It will be shown that the skulls of domestic dogs are surprisingly highly integrated when compared to the wolf although the diversity of skull shapes and frequent occurrence of orofacial disproportions implied the opposite.

The second study presented in **Chapter 2.2**, will test the hypothesis that the shape of the TMJ can be predicted from the geometry of the whole skull, which should be possible through the high integration detected in Study I. It will be shown that, because of the high integration of the skull, the diversity of TMJ shapes in dogs can, at least in part, be referred back to overall skull geometry. Yet, there are numerous deviations from this general pattern, which imply a far-reaching structural independence of the joint in the skulls of domestic dogs. In addition to that, this study is the first to capture and quantify the enormous diversity of TMJ shapes in dogs and wolves and that links variations in the TMJ to the geometry of the skull in *Canis lupus*.

In order to approach these research questions, computed tomography (CT) images were produced from more than 300 skulls of adult domestic dogs and wolves (196 of which could be used in Study I, 274 in Study II). From these images virtual 3D-reconstructions of the skulls were generated as a basis for both studies. Methods from the field of geometric morphometrics were used to analyse this data set. While in traditional morphometrics researchers relied on linear and one-dimensional measurements for their investigations, the main advantage of geometric morphometrics is the possibility to study complex shapes using statistical methods (Zelditch et al., 2012; Esteve-Altava, 2017). In geometric morphometrics, biological shape is approximated by a geometric shape which is defined by a number of landmarks (Esteve-Altava, 2017). Landmarks are named points, which are often but not necessarily anatomically homologous, that can be found on each specimen

in the sample, and that as one set adequately represent the shape under investigation (Bookstein, 1991; Zelditch et al., 2012). Depending on the quality of landmarks chosen, the results of a study may vary concerning their significance (Bookstein, 1991). After digitizing the same landmark set for all specimens in the sample, the geometric shapes captured by them needed a correction for size, orientation and location in three-dimensional space to allow analyses of pure shape. This was done by a so called procrustes superimposition, a mathematical operation that superimposes the centroid of each shape to correct for location, that rescales the centroid size (which is the sum of the squared distances of all landmarks from the centroid) to 1 to correct for size, and then minimizes the sum of squared distances between corresponding landmarks to correct for orientation (Zelditch et al., 2012). Resulting from this procedure is pure shape data (procrustes coordinates) and centroid size, which is an important measure for the size of the animal when other data such as body size or weight is not available (Zelditch et al., 2012). Subsequently, a variance covariance matrix was calculated based on the procrustes coordinates, which was the basis for all subsequent analyses. To analyse the variation in the data set, a principal component analysis (PCA) was used which ascertains all dimensions of variation and orders them according to the amount of variation they explain in the sample (Zelditch et al., 2012). In addition to that, shape disparity was calculated, which provided a measure for the diversity of shapes in the sample (Foote, 1993). In order to analyse the allometric shape component, which is the shape variation associated with varying body or skull size, centroid size was regressed on multivariate shape data (Monteiro, 1999). Geometric morphometrics also provides statistical methods to study the modularity and integration of biological structures. RV-coefficients, which are multivariate generalizations of the squared Pearson correlation coefficient, were calculated as a measure for integration strength (Fruciano et al., 2013). In order to analyse integration patterns partial least squares analyses (PLS) were performed which ascertain the main trajectories of covariation between two sets of landmarks and order them according to the amount of total covariation they explain (Zelditch et al., 2012). In these analyses strong integration is reflected by limited scattering of data points and strong adherence to a covariation trajectory, meaning that the specific shape in one structure covaries with a specific shape in the other. Vector angles and especially their significance levels, which were calculated on the basis of the PLS analyses (Klingenberg and Marugan-Lobon, 2013) were used as a measure for similarity or dissimilarity of covariation patterns between wolves and dogs, together with qualitative inference based on shape graphs. In the first study these graphs were simple wireframes, which connect the landmarks in the data set and which can be deformed on the basis of a so called “thin-

plate spline" data interpolation procedure along all PLS or PCA vectors (Bookstein, 1989). In the second study, 3D-surface models of the cranium, the mandible and the TMJ (condylar and mandibular process) were rendered and deformed analogously to make shape changes more comprehensible. As a measure for the amount of a variation which can be explained by integrated variation and not modularity, we divided the variance of PLS scores of a given structure by the sum of eigenvalues resulting from their respective PCA.

Through its artificially driven diversification, the domestic dog poses an ideal model species to study the integration and modularity of the skull and its role for radiative processes over short evolutionary time spans. In both studies it will be shown, that the evolution of new morphologies does not necessarily require a reduction of integration. Moreover, the studies will show that the patterns of variation and covariation among the skull modules not only resemble each other in domestic dogs and wolves, but also approximate patterns known for other amniotes. Thus, both studies will have implications for the evolution of form and shape way beyond the species border of *Canis lupus*.

Since this is a cumulative thesis, each of the two publications has been written to stand on its own. Redundant passages thus might occur.

2 PUBLISHED RESULTS

Study I (published online): Curth, S., Fischer, M. S., & Kupczik, K. (2017). Patterns of integration in the canine skull: an inside view into the relationship of the skull modules of domestic dogs and wolves. *Zoology*. DOI: https://doi.org/10.1016/j.zool.2017.06.002.

Disproportions in the dog skull led to the hypothesis that its integration strength and pattern was altered by artificial selection. The results however pointed to an unchanged integration of the skull modules in domestic dogs when compared to the wolf. This study and its surprising result clarified basic patterns of integration of the wolf and dog skull. It constituted the basis for a new hypothesis dealt with in Study II.

Author contributions: MSF, KK and SC conceived the study; SC sampled and CT scanned the specimens or selected existent scans, digitally reconstructed the skulls, digitized the landmarks, performed all analyses, interpreted the results, prepared the figures and wrote the manuscript. KK and MSF critically revised the manuscript and helped to finalize the article. Own contribution in total: 90 %.

Study II (published): Curth, S., Fischer, M. S., & Kupczik, K. (2017). Can skull form predict the shape of the temporomandibular joint? A study using geometric morphometrics on the skulls of wolves and domestic dogs. *Annals of Anatomy-Anatomischer Anzeiger*, 214, 53-62.

Here, it was tested whether the high integration of the skull in *Canis lupus* allows predictions concerning the shape of the TMJ based on skull shape. The results showed that the predictability of the shape of the TMJ is limited. Thus, this study qualifies the results of Study I. In spite of the generally high skull integration, small skeletal structures can gain their shape partly independently from the rest of the skull.

Author contributions: SC conceived the study, sampled and CT scanned the specimens or selected existent scans, digitally reconstructed the skulls, digitized the landmarks, performed all analyses, interpreted the results, prepared the figures and wrote the manuscript. KK and MSF critically revised the manuscript and helped to finalize the article. Own contribution in total: 90 %.

I have read the authors' contributions stated above and confirm their correctness.

Prof. Dr. Martin S. Fischer (supervisor)

2.1 Study I

Stefan Curth, Martin S. Fischer and Kornelius Kupczik

Patterns of integration in the canine skull: an inside view into the relationship of the skull modules of domestic dogs and wolves.

Published online in: *Zoology*, 2017. DOI: https://doi.org/10.1016/j.zool.2017.06.002.

Abstract

The skull shape variation in domestic dogs exceeds that of grey wolves by far. The artificial selection of dogs has even led to breeds with mismatching upper and lower jaws and maloccluded teeth. For that reason, it has been advocated that their skulls (including the teeth) can be divided into more or less independent modules on the basis of genetics, development or function. In this study, we investigated whether the large diversity of dog skulls and the frequent occurrence of orofacial disproportions can be explained by a lower integration strength between the modules of the skull and by deviations in their covariation pattern when compared to wolves. For that purpose, we employed geometric morphometric methods on the basis of 99 3D-landmarks representing the cranium (subdivided into rostrum and braincase), the mandible (subdivided into ramus and corpus), and the upper and lower tooth rows. These were taken from CT images of 196 dog and wolf skulls. First, we calculated the shape disparity of the mandible and cranium in dogs and wolves. Then we tested whether the integration strength (measured by RV-coefficient) and the covariation pattern (as analysed by Partial Least Squares analysis) of the modules subordinate to the cranium and the mandible can explain differing disparity results. We show, contrary to our expectations, that the higher skull shape diversity in dogs is not explained by less integrated skull modules. Also the pattern of their covariation in the dog skull can be traced back to similar patterns in the wolf. This shows that existing differences between wolves and dogs are at the utmost a matter of degree and not absolute.

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Patterns of integration in the canine skull: an inside view into the relationship of the skull modules of domestic dogs and wolves

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ABSTRACT

The skull shape variation in domestic dogs exceeds that of grey wolves by far. The artificial selection of dogs has even led to breeds with mismatching upper and lower jaws and malocclusion teeth. For that reason, it has been advocated that their skulls (including the teeth) can be divided into more or less independent modules on the basis of genetics, development or function. In this study, we investigated whether the large diversity of dog skulls and the frequent occurrence of orofacial disproportions can be explained by a lower integration strength between the modules of the skull and by deviations in their covariation pattern when compared to wolves. For that purpose, we employed geometric morphometric methods on the basis of 99 3D-landmarks representing the cranium (subdivided into rostrum and braincase), the mandible (subdivided into ramus and corpus), and the upper and lower tooth rows. These were taken from CT images of 196 dog and wolf skulls. First, we calculated the shape disparity of the mandible and the cranium in dogs and wolves. Then we tested whether the integration strength (measured by RV coefficient) and the covariation pattern (as analysed by partial least squares analysis) of the modules subordinate to the cranium and the mandible can explain differing disparity results. We show, contrary to our expectations, that the higher skull shape diversity in dogs is not explained by less integrated skull modules. Also, the pattern of their covariation in the dog skull can be traced back to similar patterns in the wolf. This shows that existing differences between wolves and dogs are at the utmost a matter of degree and not absolute.

1. Introduction

Domestic dog skulls (*Canis lupus f. familiaris,* Linnaeus 1758) are much more diverse than those of grey wolves and even approximate the variation found in all wild carnivores (Drake and Klingenberg, 2010). By domesticating the wolf and by breeding dogs according to breeding standards, humans created over 350 dog breeds (http://www.fci.be), each with uniquely formed heads. As a result, dog skulls vary greatly in size, relative rostrum length (from brachycephalic to dolichocephalic), skull flexion (from klinorhynch to airorhynch) and gracility/robustness (Fig. 1) (Klatt, 1949; Rosenberg, 1966; Nussbaumer, 1982; Brehm et al., 1985; Drake and Klingenberg, 2010). Moreover, some dog skulls show disproportions between the mandible and the cranium and between the teeth and the jaw (e.g., bulldogs with undershot bite and crowded teeth (Colyer, 1990), implying a reduced integration of those skull parts when compared to the wolf.

Parts of the skull which are strongly integrated internally but only weakly integrated with other skull parts have been coined 'modules' (Klingenberg, 2008, 2009, 2013). For example, some authors have suggested that the rostrum and the braincase are modules of the dog cranium (Drake and Klingenberg, 2010; Cardini and Polly, 2013). A modular structure like this could allow single modules to vary more independently without any negative consequences for other parts of the skull. This would then, in theory, increase the evolvability of the skull as a whole (Goswami, 2007; Zelditch et al., 2008; Kuratani, 2009; Marroig et al., 2009; Porto et al., 2009, 2013). Modularity in the skull is suggested to be caused by 'local factors'. These are influences, such as specific genes or developmental factors, which affect only restricted areas of the skull rather than the skull as a whole (Mitteroecker and Bookstein, 2008). For example, certain genes influence tooth formation without any direct effects on the surrounding bone (Hall, 2003).

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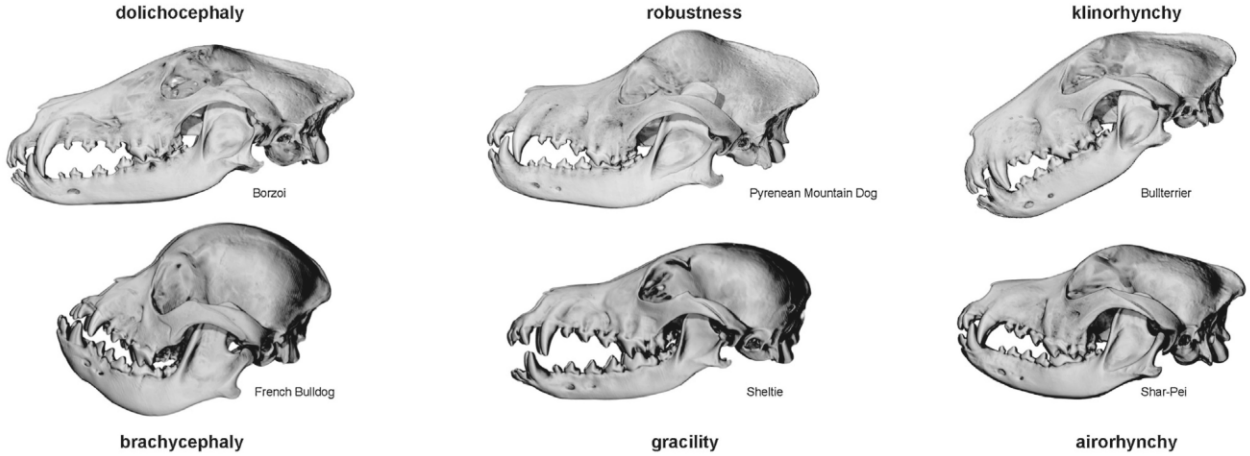

Fig. 1. Three commonly seen types of shape variation in the dog skull: relative rostrum length, robustness and skull flexion exemplified by six representative breeds.

On the other hand, the modules of the skull can never be fully independent from one another. This is guaranteed by their integration in the skull which is generated by 'common factors', which have an effect on the whole skull rather than on separate modules (Mitteroecker and Bookstein, 2008). Such factors are pleiotropic genes, shared developmental pathways or a common function, which underlies similar environmental influences such as diet. These common factors can also account for variation in the skull, but rather than allowing individual modules to vary independently, common factors cause multiple modules or even the whole skull to vary. For example, size variation in mammals causes variation throughout the whole skull rather than in restricted areas (Cardini and Polly, 2013).

In the present study we were interested in the balance of integration and modularity in the skulls of wolves and dogs. We assumed that less integrated skull modules could provide an explanation for the enormous skull diversity and frequent occurrence of orofacial disproportions in dogs, as opposed to the highly integrated and less diverse wolf skulls. Specifically, we hypothesized (i) that the shape disparity of the mandible and cranium (both with and without the dentition) would be significantly larger in dogs than in wolves, (ii) that this larger disparity would be explained by a low integration strength of the modules subordinate to the cranium and the mandible, and (iii) that the covariation pattern of these modules would also be different in dogs and wolves, giving another explanation for the larger skull shape variation in dogs which in part lacks natural equivalents.

The balance of integration and modularity and the covariation patterns of modules can be tested and described by using statistical methods from the field of geometric morphometrics. We did this on the basis of 196 CT scans of dog and wolf skulls and a data set of 99 landmarks. These define the shapes of several exemplary modules in the dog skull, among them the rostrum and the braincase of the cranium, the corpus and ramus of the mandible and the upper and lower tooth row. We decided on those modules based on numerous findings by other authors suggesting partly separate genetic determination and inheritance of those skull parts but also diverging selective pressures (e.g., Grüneberg and Lea, 1940; Stockard, 1941; Herre, 1951; Starck, 1953; Stengel, 1958; Boughner and Hallgrímsson, 2008; Drake and Klingenberg, 2010; Cramon-Taubadel, 2011; La Croix et al., 2011; Gómez-Robles and Polly, 2012; Le Cabec et al., 2012; Asahara, 2013).

2. Materials and methods

2.1. Sample

Our sample (Table S1 in the online Appendix) comprised 140 skull CT scans of domestic dogs (adult, both sexes, 103 breeds, types or mixtures) and 56 scans of grey wolves (adult, both sexes, different subspecies originating from North America, Europe and Asia). We aimed at covering a wide spectrum of skull forms, both in wolves and dogs. We obtained the skulls from different museums and scanned them with computerized tomography (CT) at multiple locations (details are given in Tables S1 and S2). During the scans, the mandible was articulated to the cranium.

2.2. 3D-reconstructions and landmarks

We volume-rendered the skulls in 3D in Avizo v. 7 (FEI Visualization Sciences Group, Mérignac, France) and placed a set of 99 landmarks (LM) on the renderings or directly on the CT slices (Fig. 2 and Table S3). We chose the landmarks to sufficiently reflect the external shape of the cranium and the mandible and to capture the spatial position and orientation of the teeth (including the tooth roots). We solely obtained the landmarks for the left half of the skull to reduce the total number of landmarks. In the case of missing landmarks on this side, we obtained all landmarks from the right side of the skull and mirrored them for the analysis. The procedure of digitizing only one half of a bilateral structure is applicable to dogs and wolves because their skull shape variation approaches macroevolutionary levels (Drake and Klingenberg, 2010; Cardini, 2016). Only one researcher (S.C.) digitized the landmarks in order to eliminate interobserver errors. Moreover, we performed a Procrustes ANOVA on a subset of the sample (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002) to calculate the intraobserver error during the landmark digitization. For each specimen the landmarks were digitized three times on the same CT scan. The results reflected that the error of the landmark digitization is very low compared to the skull shape variation in the sample (for the results see Table S4).

2.3. Geometric morphometric analysis

We imported the landmark set into MorphoJ (Klingenberg, 2011) and divided it into four different sets of landmarks: the mandible (13 LM), the mandible including teeth (42 LM), the cra-

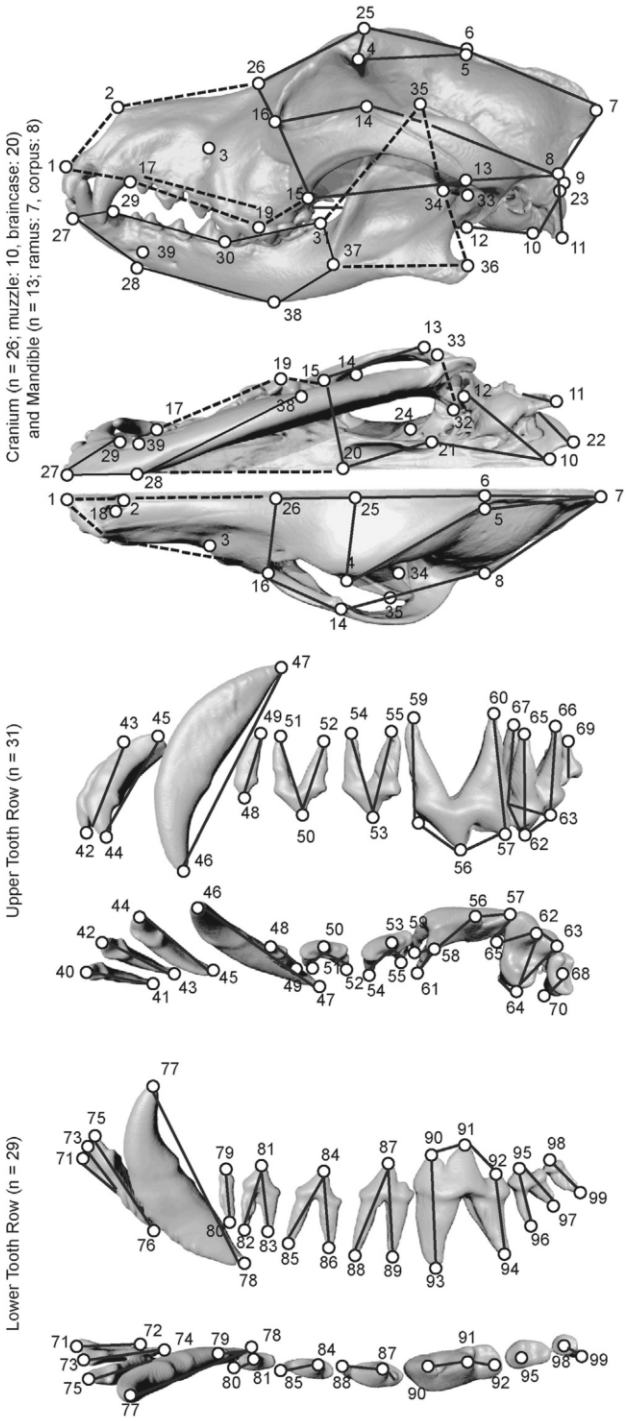

Fig. 2. Position of the 99 3D-landmarks on the cranium, the mandible and the upper and lower tooth row. For landmark definitions see Table S3 in the online Appendix. The wireframe graphs show the subdivision of the cranium and the mandible into an anterior and posterior portion and will be used to visualize shape deformations in Figs. 4–7.

nium (26 LM) and the cranium including teeth (57 LM). We used these sets later for the partial least squares (PLS) analyses. Since separate landmark sets are required for the calculation of the shape disparity and the integration strength, we subdivided the sets further into the rostrum (10 LM) and braincase (20 LM, 4 LM shared with rostrum), the ramus (7 LM) and corpus (8 LM, 2 LM shared with ramus) of the mandible, and the upper tooth row (31 LM) and lower tooth row (29 LM). The basic modular structure of the cranium and the mandible used in the present study was inspired by studies such as those by Drake and Klingenberg (2010), Meloro and Slater (2012) and Piras et al. (2013), although more complex modularity scenarios for both structures have been proposed by now (Cheverud et al., 1991; Goswami and Polly, 2010; Anderson et al., 2014; Parr et al., 2016). We analyzed the dentition as a separate module apart from the mandible and the cranium in an exploratory way, since previous research had shown that dental traits are not necessarily correlated to the rest of the skull (Dayan et al., 2002; Cobourne and Sharpe, 2003; Boughner and Hallgrímsson, 2008; Gómez-Robles and Polly, 2012; Le Cabec et al., 2012; Asahara, 2013). We performed Procrustes fitting on all these data sets separately in order to eliminate differences in orientation, size and location, resulting in pure shape data (Procrustes coordinates). For the PLS analysis the two modules in question were jointly fitted to conserve their relative position and size. Afterwards, we subdivided each data set into separate sets for wolves and dogs to allow subsequent comparisons between both groups.

We then analyzed the shape disparity of the cranium and the mandible, both including and excluding the teeth to test whether both parts are more diverse in dogs than in wolves. We did this analysis with Disparity Box 3D which is included in the IMP series (H. David Sheets, Dept. of Physics, Canisius College, Buffalo, NY, USA). We then analyzed the integration strength between the cranium and the upper tooth row, the mandible and the lower tooth row, the rostrum and the braincase, and the ramus and corpus of the mandible. We did this to test whether a lower integration strength of those modules explains the larger diversity of the mandible and cranium in dogs. We performed this analysis in the RV comparison tool (Fruciano et al., 2013), which calculates an RV coefficient as a measure for the strength of integration and a *p*-value indicating the significance of any calculated differences between wolves and dogs. We also tested for the correlation (Pearson's *r*) of RV coefficients and disparity values. Then we compared the integration pattern of the above-mentioned module pairs between wolves and dogs. We did this in order to detect deviations from the ancestral (wolf-like) integration pattern in dogs, which could explain their higher skull shape disparity. For that purpose we performed multiple PLS analyses in MorphoJ separately for wolves and dogs (Rohlf and Corti, 2000; Klingenberg, 2013). We qualitatively described our observations on the basis of wireframe graphs generated in MorphoJ and calculated the angles between the PLS vectors. Significant vector angles indicate vector resemblance regardless of angle magnitude (Klingenberg and Marugan-Lobon, 2013). Moreover, we calculated the variance of every module that is explained by the first four PLS vectors by dividing the variance of PLS scores by the total variance of the module. In every case the PLS axes that follow PLS 2 explained less than 5% of module variance. Thus we concentrated on PLS 1 and 2 for the analysis. In order to ensure that allometric effects on skull shape are similar in both dogs and wolves and are hence negligible for the present study, we regressed the centroid size of the mandible and the cranium on their shape separately for both groups. We then compared the shape changes associated with size qualitatively and calculated the vector angles between wolves and dogs. Moreover, we checked for correlations of centroid size and PLS scores. We also tested the sexual dimorphism of dog and wolf skulls via discriminant function. The skulls of wolves and dogs respond in similar ways to size changes and show similar signs of sexual dimorphism. Thus, these two factors should be of minor importance for the present study. For the sake of brevity, we put the detailed results in the supplementary online Appendix (datasheets S5 and S6).

Table 1
The integration strength (RV coefficient) of each module combination in dogs and wolves.

	Module 1	Module 2	RV Dogs	RV Wolves	Difference	P (1000 perm.)
Mandible	Ramus	Corpus	0.233	0.281	–0.048	0.63
Mandible (incl. teeth)	Mandible	Lower dentition	0.485	0.437	0.049	0.692
Cranium	Braincase	Rostrum	0.665	0.511	0.154	0.222
Cranium (incl. teeth)	Cranium	Upper dentition	0.737	0.429	0.308	0.018

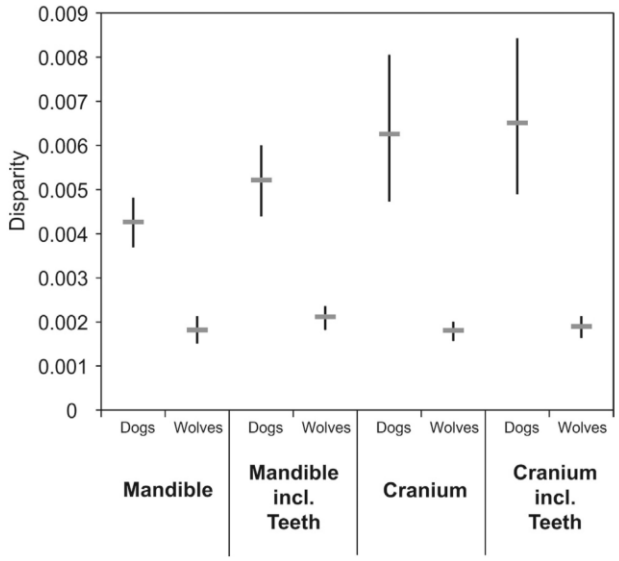

Fig. 3. The shape disparity of the cranium and the mandible (both with and without the teeth) in wolves and dogs. The vertical bars represent 95% confidence intervals. In every instance domestic dogs are more diverse than wolves.

3. Results

3.1. Skull shape disparity in wolves and dogs

The shape disparity tests resulted in the values given in Fig. 3. Each of the skull parts tested (mandible excluding and including the teeth, cranium excluding and including the teeth) was more diverse in dogs than in wolves. This supported our first hypothesis of an overall higher skull shape disparity in dogs when compared to wolves.

3.2. Skull integration strength in wolves and dogs

The RV coefficients of the modules subordinate to the mandible and cranium are shown in Table 1. The ramus and corpus of the mandible, the mandible and the lower tooth row, and the rostrum and braincase in the cranium were not significantly more strongly integrated in wolves than in dogs ($p > 0.05$). The cranium and the upper tooth row were significantly stronger integrated in dogs than in wolves ($p = 0.018$). Testing the correlation of RV coefficients and disparity values resulted in no significant correlation for wolves ($r = 0.16$; $p = 0.8354$), a significant correlation for dogs ($r = 0.995$; $p = 0.0046$) and no significant correlation when both groups were analyzed together ($r = 0.6208$; $p = 0.1005$).

Our second hypothesis had to be rejected: the skull modules in dogs are not less integrated than in wolves. The integration strength in wolves is either not significantly different or, in one case, even lower than in dogs. Moreover and surprisingly, higher integration strength of the modules is associated with larger disparity of the mandible and the cranium in dogs.

Table 2
Angles between PLS vector 1 and 2 for the covariation of ramus and corpus in wolves and dogs. Significant angles are given in bold print.

Angle (°)		Wolves	
		PLS1	PLS2
Dogs	PLS1	**60**	**63.4**
	PLS2	**54.6**	**67.8**
p-value		Wolves	
		PLS1	PLS2
Dogs	PLS1	**0.00307**	**0.009**
	PLS2	**0.00041**	**0.02998**

Table 3
Angles between the PLS vectors 1 and 2 for the covariation of rostrum and braincase in dogs and wolves. Significant angles are given in bold print.

Angle (°)		Wolves	
		PLS1	PLS2
Dogs	PLS1	85	**45**
	PLS2	**38.4**	77.2
p-value		Wolves	
		PLS1	PLS2
Dogs	PLS1	0.46757	**<0.00001**
	PLS2	**<0.00001**	0.06121

3.3. Skull integration patterns in wolves and dogs

3.3.1. Covariation of the modules subordinate to the cranium and the mandible

Fig. 4 shows the integration pattern of the mandibular ramus and corpus of dogs and wolves. Similar patterns characterized both sides in PLS 1 and 2 as indicated by highly significant vector angles (Table 2) and similar shape features (Fig. 4). Yet the amount of covariation they explain differed between both groups. The covariation of the mandibular ramus and corpus under varying gracility/robustness (see Fig. 1) of the skull explained 51.6% of the covariation in wolves (PLS 1, $p = 0.001$; covers 3.1% of ramus variance and 7.3% of corpus variance; see datasheet S7 in the supplementary online Appendix). Yet in dogs it only explained 7.9% of it (PLS 2, $p < 0.0001$; covers 4.8% of ramus variance and 4.1% of corpus variance: S7; angle: 54.6°, $p = 0.00041$). In wolves, 24.6% of the integration pattern was explained by the covariation of relative mandibular ramus height and the curvature of the mandibular corpus, a feature that is connected to relative rostrum length (PLS 2, $p = 0.0005$; covers 2.4% of ramus variance and 5.9% of corpus variance: S7). In dogs, this explained 87.0% of the covariation (PLS 1, $p < 0.0001$; covers 24.8% of ramus variance and corpus variance: S7; angle between both groups: 63.4°, $p = 0.009$).

Fig. 5 shows the basic integration pattern of the rostrum and braincase in the cranium in PLS 1 and 2. Again, similar patterns characterized the integration in wolves and dogs as shown by significant vector angles (Table 3) and similarities in the shape graphs (Fig. 5), but with varying predominance of specific directions of covariation. While in wolves 40.6% of the covariation was explained by the degree the rostrum is flexed relative to the braincase (PLS 1, $p < 0.0001$; covers 7.4% of braincase variance and 3.9% of rostrum variance: S7), in dogs it accounted only for 2.9% (PLS 2, $p = 0.0110$;

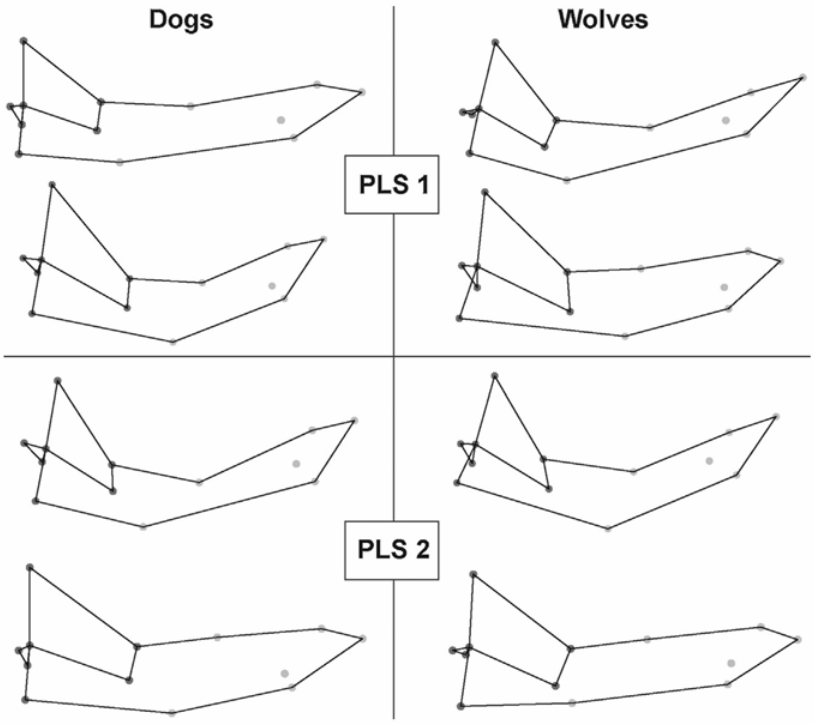

Fig. 4. The integration pattern of ramus (dark grey) and corpus (light grey) in the mandible in two PLS analyses for wolves and dogs. Each box contains the wireframe graphs scaled to positive and negative PLS values along each axis, to the left for dogs and to the right for wolves. All subsequent figures containing wireframe graphs were structured in the same manner.

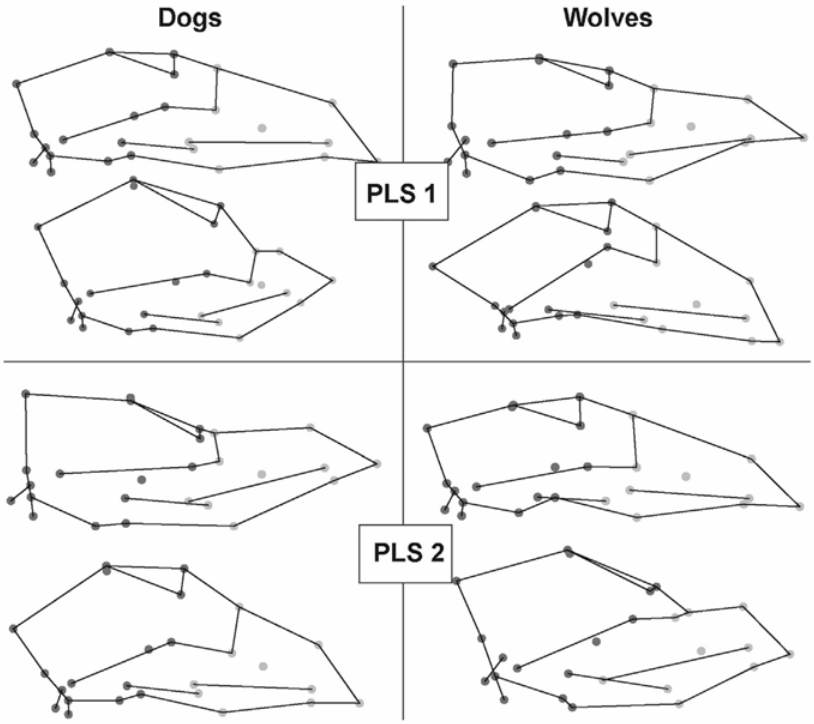

Fig. 5. The integration pattern of rostrum (light grey) and braincase (dark grey) in the cranium in two PLS analyses for wolves and dogs.

covers 7.5% of braincase variance and 2% of rostrum variance: S7; angle: 38.4°, $p < 0.00001$). While in wolves 30.4% of the covariation was explained by the relative length of the rostrum with regard to the braincase (PLS 2, $p < 0.0001$; covers 4.9% of braincase variance and 4.7% of rostrum variance: S7), in dogs it accounted for most of the covariation (PLS1, 95.3%, $p < 0.0001$; covers 23.9% of braincase variance and 16.2% of rostrum variance: S7; angle: 45°, $p < 0.00001$).

3.3.2. *Covariation of the teeth and the mandible and the cranium*

Fig. 6 shows the integration of the mandible and the lower tooth row in wolves and dogs, Table 4 shows the vector angles between

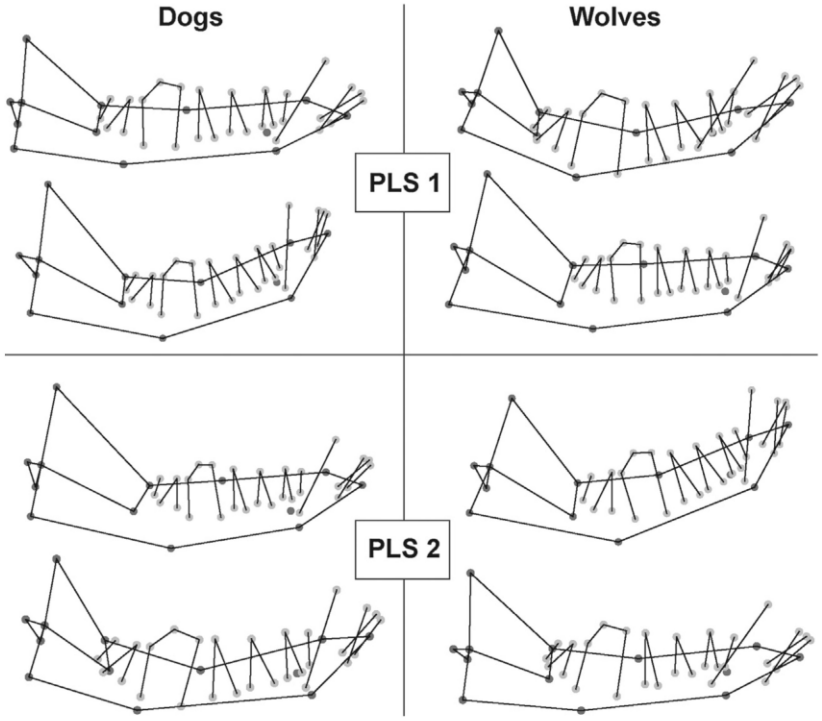

Fig. 6. The integration pattern of the mandible (dark grey) and the lower tooth row (light grey) in two PLS analyses for wolves and dogs.

Table 4
Angles between the PLS vectors 1 and 2 for the covariation of mandible and lower tooth row in wolves and dogs. Significant angles are given in bold print.

Angle (°)		Wolves	
		PLS1	PLS2
Dogs	PLS1	88.1	**42**
	PLS2	**38.5**	86.1
p-value		Wolves	
		PLS1	PLS2
Dogs	PLS1	0.71442	**<0.00001**
	PLS2	**<0.00001**	0.45945

Table 5
Angles between the PLS vectors 1, 2 and 3 for the covariation of cranium and upper tooth row in dogs and wolves. Significant angles are given in bold print.

Angle (°)		Wolves		
		PLS1	PLS2	PLS3
Dogs	PLS1	**77.3**	**42.9**	**79.5**
	PLS2	83.8	**69.7**	**72.9**
	PLS3	**41.3**	87.8	**75.4**
p-value		Wolves		
		PLS1	PLS2	PLS3
Dogs	PLS1	**0.00451**	**<0.00001**	**0.01928**
	PLS2	0.167	**<0.00001**	**0.00012**
	PLS3	**<0.00001**	0.62023	**0.00107**

both groups. Again, the covariation was characterized by similar patterns in both wolves and dogs. But again, the amount of the covariation spectrum explained by each direction of covariation differed between both groups. In wolves, PLS 1 reflected the covariation of relative tooth size and mandible size. It explained 58.3% of the covariation ($p < 0.001$; covers 10% of mandible variance and 13% of lower tooth row variance: S7). The same covariation pattern was found in dogs in PLS 2, where it accounted only for 14.6% of the total covariation ($p < 0.0001$; covers 9.2% of mandible variance and 8.8% of lower tooth row variance: S7; angle: 38.5°, $p < 0.00001$). PLS 1 in dogs was explained to 80.5% by a covariation of dorso-ventral mandible curvature and tooth row curvature ($p < 0.0001$; explains 22.3% of mandible variance and 21.2% of lower tooth row variance: S7). The same pattern in wolves explained 14.7% of the covariation (PLS 2, $p < 0.001$; covers 6.7% of mandible variance and 5.4% of lower tooth row variance: S7; angle: 42°, $p < 0.00001$).

Fig. 7 shows the integration pattern of the cranium and the teeth in wolves and dogs, Table 5 shows the vector angles between both groups. In wolves, PLS 1 explained 40.7% of the covariation ($p < 0.001$; covers 8.5% of cranium variance and 4.9% of the variance of the teeth: *S7*). It was characterized by cranial variation in skull flexion that covaried with varying molar size and procumbence of the anterior teeth. The same covariation pattern was found in dogs in PLS 3 (1.8%, $p < 0.001$; covers 4.9% cranium variance and 4.2% of upper dentition variance: S7), and it was also indicated by a highly significant vector angle (41.3°, $p < 0.00001$). In wolves, PLS 2 explained 29.1% of the covariation ($p < 0.001$; covers 7.3% of cranium variance and 5% of upper dentition variance: S7). It was characterized by varying relative rostrum lengths that covary with different degrees of tooth crowding and curvature of the tooth row. The same pattern was found in dogs where it explained 95.6% of the whole covariation (PLS 1, $p < 0.001$; covers 31.2% of cranium variance and 25.7% of upper dentition variance: S7; angle between both groups: 42.9°, $p < 0.00001$). In wolves, PLS 3 accounted for 8.7% of the covariation pattern ($p = 0.001$; covers 4.7% of cranium variance and 2.7% of upper dentition variance: S7). It was characterized by a variation in cranial gracility connected to varying molar (crown and root) size and anterio-posterior position of the incisors. A similar pattern was found in PLS 2 of dogs where cranial gracility also covaries with relative tooth size, yet not significantly (2.1% of the covariation, $p = 0.102$; covers 6.1% of cranium variance and 4% of upper dentition variance: S7).

In sum, similar covariation patterns characterized the integration of all skull modules tested in wolves and dogs when the

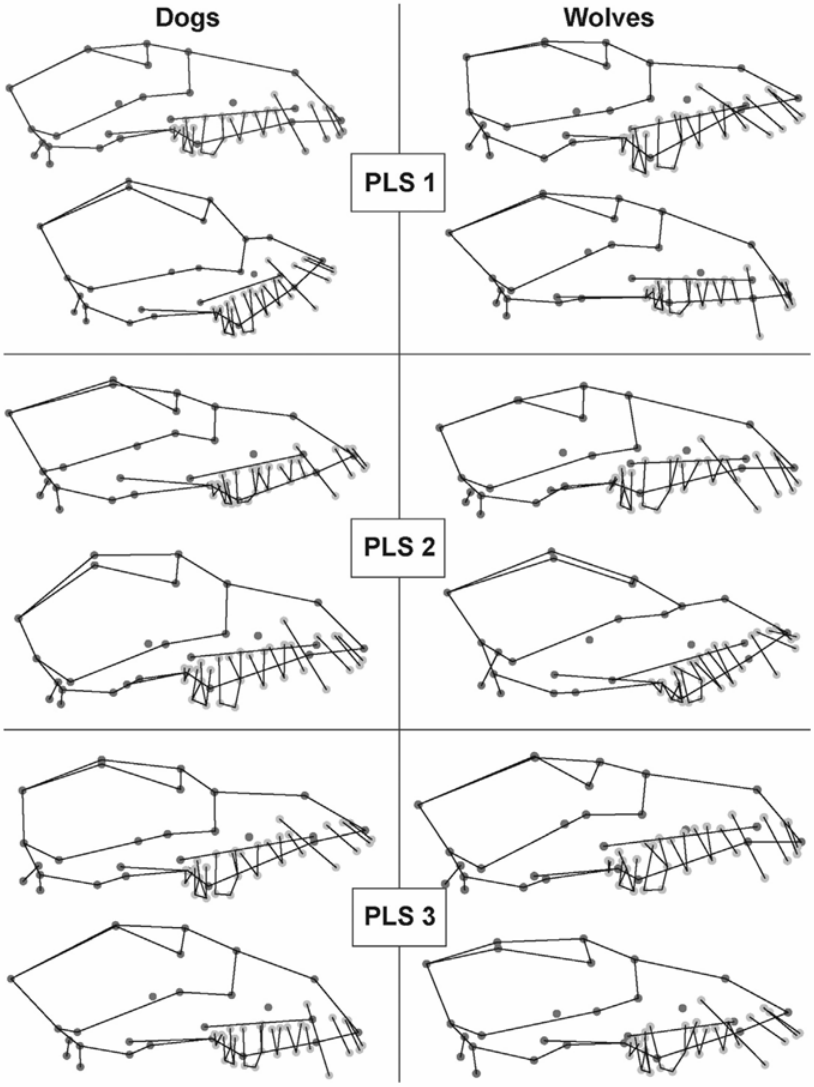

Fig. 7. The integration pattern of the cranium (dark grey) and the upper tooth row (light grey) in two PLS analyses for wolves and dogs.

wireframe graphs were used for interpretation and vector angles were considered. In both groups the patterns were heavily influenced by varying degrees of skull flexion and relative rostrum length but also varying gracility of the skull. However, the integration pattern in dogs and wolves differed in two ways: (i) The amount of covariation (and module variance) explained by each type of variation (skull flexion, rostrum length etc.). In dogs the variation of relative rostrum length determined most of the covariation spectrum (in PLS 1), while in wolves the variation in skull flexion or varying gracility was predominant. (ii) In wolves the disparity within each type of variation was smaller than in dogs. Hence the wireframe graphs in each visualisation of wolves needed to be exaggerated in order to make the shape variation visible. In dogs, basically the same shape variation as in wolves was much more distinct.

4. Discussion

In the present study, we were interested in the integration of six skull modules in wolves and dogs. In view of the enormous diversity of dog skull shapes, which often include orofacial disproportions (Colyer, 1990), we hypothesized that much of this variation might be caused by a lower integration strength of the skull modules and deviations from the wolf-like skull integration pattern. We tested this hypothesis with a data set of 140 dogs and 56 wolves using geometric morphometrics on shape data of four exemplary module pairs: the ramus and corpus of the mandible, the rostrum and braincase of the cranium, the upper tooth row and the cranium and the lower tooth row and the mandible.

The enormous disparity of the dog cranium, which is close to that of all carnivores, has been quantified before (Drake and Klingenberg, 2010; Parr et al., 2016). Nonetheless, we repeated the calculation of cranial shape disparity in conjunction with the mandible and the dentition. By doing this, we provided the evidence that dogs are significantly more diverse in these skull parts than wolves (Fig. 3), which reinforced the assumption from which we started this study. Then we tested the integration strength of the modules subordinate to the mandible and cranium. Surprisingly, none of the tested module pairs was less integrated in dogs than in wolves (Table 1). The integration strength between the corpus and ramus of the mandible, the rostrum and the braincase of the cranium, and the lower tooth row and the mandible was not significantly different in wolves and dogs. The integration strength of the cranium and upper tooth row was even higher in dogs than in

wolves. This emphasizes the dependency of the dental arch shape on the surrounding skull. These results suggest that lower integration strengths of skull modules (e.g., of ramus and corpus) do not necessarily explain higher shape disparity in superordinate skeletal structures (e.g., the mandible). Specifically for dogs, it means that artificial selection did not reduce the integration of the skull. Whether modularity increases the shape disparity and also the evolvability of biological structures is still under debate (Goswami and Polly, 2010; Sanger et al., 2012; Parr et al., 2016). Our results, however, suggest that the variation of the dog skull primarily arose not from the increased semi-independent variation of skull modules, but rather from variation of the skull as a whole. Our results even point to an association of higher integration strength with larger disparity at least in dogs, which opposes our initial assumption. Thus, our results are closer to those of Goswami and Polly (2010) who concluded, based on their analysis of a sample of primates and carnivores, that modularity does not facilitate shape disparity and evolvability. On the other hand, Parr et al. (2016) came to the opposite conclusion in their study on dingoes and domestic dogs. Clearly, more studies are needed to understand the relationship of modularity and integration and its consequences for the evolution of biological structures.

We also compared the patterns of covariation of the skull modules in dogs and wolves. Although the different module sets underwent separate PLS analyses, all of them pointed to a potential influence of similar types of skull shape variation on the covariation pattern, as inferred both from visual inspection of the shape graphs and the significance of vector angles between the groups. These types of variation were varying relative rostrum lengths, skull flexion and varying gracility or robustness. Variation in regard to these characteristics is well known in dogs and has been described before by numerous authors (McKeown, 1975; Brehm et al., 1985; Drake and Klingenberg, 2010; Georgevsky et al., 2014). Where these types of shape variation had an influence on the covariation pattern, the way the mandibular and cranial modules covaried was very similar in both wolves and dogs as shown via wireframe shape graphs. These patterns resemble the variation of the skull in a wide range of mammalian taxa, suggesting the conservation of ancestral integration patterns even in domestic animals. Variation and covariation which is based on relative rostrum lengths, for instance, has been reported for humans and great apes, antelopes, bats, rodents, mongooses and other carnivores and even marsupials (Wroe and Milne, 2007; Hautier et al., 2012; Singh et al., 2012; Cardini and Polly, 2013; Figueirido et al., 2013; Cardini et al., 2015). Skull flexion or robustness have been described as variable shape features with an influence on the covariation patterns within the skull in carnivores and rodents (Radinsky, 1981; Sears et al., 2007; Hautier et al., 2012; Asahara, 2013; Figueirido et al., 2013). These recurring patterns also imply the conservation of underlying factors, such as developmental pathways or pleiotropic genetic determination (Goswami, 2006; Mitteroecker and Bookstein, 2008; Marroig et al., 2009; Porto et al., 2009; Drake and Klingenberg, 2010; Singh et al., 2012; Figueirido et al., 2013; Goswami et al., 2014). Mutations in the genes coding for bone morphogenetic proteins, for instance, have been shown to correlate with facial length in domestic dogs (gene BMP3) (Schoenebeck et al., 2012) and even birds (gene BMP4) (Wu et al., 2004). Canine klinorhynchy and facial length are strongly associated with mutations in regions coding for the runt-related transcription factor 2 (Runx-2) (Fondon and Garner, 2004, 2007; Sears et al., 2007) and robust and gracile skull shape features are associated with a variation in body size, which in dogs is controlled by a variety of genes (Rimbault et al., 2013) and the allometric scaling of the brain and musculature (Radinsky, 1981; Penrose et al., 2016).

Aside from these similarities, our results also point to some differences in the covariation pattern between wolves and dogs. In the wireframe graphs of our study, differences between wolves and dogs are visible in the size of the orbits, which is less affected by rostrum shortening in dogs than in wolves, and the generally more domed foreheads in dogs (; dogs: PLS 1, wolves: PLS 2). Another difference between wolves and dogs was the amount to which the whole covariation spectrum was explained by each type of variation. For the skull modules in wolves, the variation in skull flexion accounted for most of the covariation in the cranium. The variation in gracility or robustness explained most of the covariation of mandibular modules in wolves. In dogs, however, the influence of relative rostrum length on the covariation of head modules was predominant. The artificial selection of the dog was obviously more focused on variations in relative rostrum length, although skull flexion is a main source for skull variation in wild wolves (Milenković et al., 2010). Finally, dogs also differed from wolves in the markedness of specific shape features. In all cases, the shape features presented in Figs. 4–7 were much more distinct in dogs than in wolves in each direction of covariation. Starting from a wolf-like medium condition, the spectrum of covariation in domestic dogs extends at both ends for every type of shape variation (e.g., shorter *and* longer relative rostrum length, more *and* less robust skulls). Since these types of variation are most likely generated by quantitative genetic effects, this suggests that breeders unconsciously selected for varying degrees of expression of the genes underlying each shape type of variation (Fondon and Garner, 2004, 2007; Wu et al., 2004). Similar ways to generate novel phenotypes are also likely for wild animals such as great apes, where orangutans, although deviating in their skull morphology through airorhynchy, share the same general integration pattern as other great apes and humans (Singh et al., 2012).

5. Conclusion

In highly integrated structures, such as the mammalian skull, morphological novelties are more likely to arise by exploitation of ancestral patterns, which can be conserved over large evolutionary time scales (Singh et al., 2012; Goswami et al., 2014). Our results imply that although the domestic dog has immensely diverse head shapes, the diversification of the dog under artificial selection also relied on the exploitation of ancestral (wolf-like) covariation patterns between the cranial and mandibular modules and the teeth. We assume that, guided by breeding standards, breeders selectively bred unusual phenotypes and increased foremost the variation of the dog skull with regard to rostrum length by unconsciously selecting for different degrees of gene expression. Existing differences in the integration of the skull between wolves and dogs are by no means stark or absolute, but rather a matter of degree.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.zool.2017.06.002.

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2.2 Study II

Stefan Curth, Martin S. Fischer and Kornelius Kupczik

Can skull form predict the shape of the temporomandibular joint? A study using geometric morphometrics on the skulls of wolves and domestic dogs.

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Abstract

The temporomandibular joint (TMJ) conducts and restrains masticatory movements between the mammalian cranium and the mandible. Through this functional integration, TMJ shapes in wild mammals are strongly correlated with diet, resulting in a wide range of TMJ variations. Yet in artificially selected and closely related domestic dogs, dietary specialisations between breeds can be ruled out as a diversifying factor. Nonetheless they display an enormous variation in TMJ shapes. This raises the question of the origin of this variation. Here, we hypothesised that TMJ shape can be predicted by skull form, i.e. that the TMJ is highly integrated in the dog skull despite reduced functional demands. If true, TMJ variation in the dog would be a plain by-product of the enormous cranial variation in dogs and its genetic causes. We addressed this hypothesis by using geometric morphometrics on a data set of 274 dog and wolf skulls. We digitized 53 three-dimensional landmarks of the skull and the TMJ on CT-based renderings and tested (1) the variation of domestic dog and wolf TMJs (via principal component analysis) and (2) the pattern of covariation of skull size, flexion and muzzle length with TMJ shape (via regression of centroid size on shape and partial least squares analyses). We show that the TMJ in domestic dogs is significantly more diverse than in wolves. Its shape covaries significantly with skull size, flexion and muzzle proportions in patterns which are similar to those observed in primates. Similar patterns in carnivorous dogs and wolves and these, mostly frugivorous, mammals imply the existence of basic TMJ integration patterns which are independent of dietary adaptations. Yet, only limited amounts of the TMJ variation in dogs can be explained by simple covariation with overall skull geometry. This implies that the final TMJ shape is gained partially independent of the rest of the skull.

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RESEARCH ARTICLE

Can skull form predict the shape of the temporomandibular joint? A study using geometric morphometrics on the skulls of wolves and domestic dogs

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ABSTRACT

The temporomandibular joint (TMJ) conducts and restrains masticatory movements between the mammalian cranium and the mandible. Through this functional integration, TMJ morphology in wild mammals is strongly correlated with diet, resulting in a wide range of TMJ variations. However, in artificially selected and closely related domestic dogs, dietary specialisations between breeds can be ruled out as a diversifying factor although they display an enormous variation in TMJ morphology. This raises the question of the origin of this variation. Here we hypothesise that, even in the face of reduced functional demands, TMJ shape in dogs can be predicted by skull form; i.e. that the TMJ is still highly integrated in the dog skull. If true, TMJ variation in the dog would be a plain by-product of the enormous cranial variation in dogs and its genetic causes. We addressed this hypothesis using geometric morphometry on a data set of 214 dog and 60 wolf skulls. We digitized 53 three-dimensional landmarks of the skull and the TMJ on CT-based segmentations and compared (1) the variation between domestic dog and wolf TMJs (via principal component analysis) and (2) the pattern of covariation of skull size, flexion and rostrum length with TMJ shape (via regression of centroid size on shape and partial least squares analyses). We show that the TMJ in domestic dogs is significantly more diverse than in wolves: its shape covaries significantly with skull size, flexion and rostrum proportions in patterns which resemble those observed in primates. Similar patterns in canids, which are carnivorous, and primates, which are mostly frugivorous imply the existence of basic TMJ integration patterns which are independent of dietary adaptations. However, only limited amounts of TMJ variation in dogs can be explained by simple covariation with overall skull geometry. This implies that the final TMJ shape is gained partially independently of the rest of the skull.

1. Introduction

The size and shape of the temporomandibular joint (TMJ) is an important factor determining masticatory function (Wall, 1999) in addition to dental occlusion (Smits and Evans, 2012), the forces exerted by the masticatory muscles (Dessem, 1989; Dessem and Druzinsky, 1992), movement at the mandibular symphysis (Scapino, 1965; Scapino, 1981; Hylander, 1985) and overall skull geometry (Vollmerhaus and Roos, 1996; Figueirido et al., 2013; Meloro et al., 2015). Thus, mammals have evolved a wide variety of TMJ shapes which reflect dietary adaptations (Moffett, 1966; Smith et al., 1983; Taylor, 2005; Srivastav and Singh, 2013; Terhune, 2013a; Terhune et al., 2015). The TMJ of carnivores is adapted to high biting force (Scapino, 1965; Dessem and Druzinsky, 1992; Crompton et al., 2006). A cylindrical, medio-laterally extended condylar process at the approximate height of the occlusal plane articulates with the cranium in a mandibular fossa, which encompasses the condylar process by a strong retroarticular process (Scapino, 1965; Gorniak, 1986; Dessem and Druzinsky, 1992). While these features apply to the grey wolf (*Canis lupus*), they can be profoundly altered in domestic dogs. This leads to a large variety in dog TMJ morphology, both within and between breeds (Ström et al., 1988; Macready et al., 2010; Dickie and Sullivan, 2001; Stewart et al., 1975; Robins and Grandage, 1977; Gibbs, 1977; Johnson, 1979; Hoppe and Svalastoga, 1980; Vollmerhaus and Roos, 1996; Dickie et al., 2002; Schwarz et al., 2002; Lerer et al., 2014; Villamizar-Martinez et al., 2016).

Different dietary adaptations among dog breeds as a cause for TMJ variation is highly unlikely under human husbandry. Because

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of the provision of food by humans, the functional demands on the dog TMJ are very much reduced. Previous authors proposed brachycephaly, undershot bites, chondrodystrophy or weakness at the mandibular symphysis as explanations for the TMJ variation in dogs (Vollmerhaus and Roos, 1996; Dickie et al., 2002). However, most of these factors were left untested (Schwarz et al., 2002), leaving the question of the causes of dog TMJ variation unanswered. In domesticated dogs, mutations in regions of the genome regulating certain growth factors (such as BMP3, RUNX2 and insulin-like growth factors) have been shown to result in a wide range of rostrum proportions, degrees of skull flexion and skull sizes (Fondon and Garner, 2004; Schoenebeck et al., 2012; Rimbault et al., 2013). In a previous study, we have shown that the dog skull is surprisingly highly integrated, although the diversity of shapes (among them bulldogs with undershot bites), implied a reduced integration strength (Curth et al., 2017). This result gave rise to the idea that the variation in the dog TMJ might be a simple by-product of skull shape variation. Consequently, this study aims to demonstrate the variation in canine TMJ before testing whether the geometry of the skull can predict TMJ shape. Assuming a high integration of the skull and the TMJ, genetic variations limiting the size and facial growth of the skull will also limit bone formation at the TMJ. Specific skull forms should therefore covary with specific TMJ forms if both are influenced by the same growth factors. Here we tested the hypothesis that large, dolichocephalic and klinorhynch skulls will have more robust TMJs (with a voluminous condylar and retroarticular process) due to overall increased bone growth while small, brachycephalic and airorhynch skulls will covary with reduced TMJs (with a small or lacking retroarticular process and a slender condylar process).

We addressed this hypothesis with methods from the field of geometric morphometrics. Using 53 three-dimensional landmarks, we captured the shape of the cranium, mandible and TMJ of 214 dogs and 60 wolves on 3D-reconstructed skulls based on computed-tomography (CT) scans. In contrast to traditional morphometrics, which relies on linear measurements or angles, geometric morphometrics allows the comparison of complex shapes. This, in our view, is crucial when studying TMJ morphology, since features such as the convexity or concavity of the joint surfaces are difficult to capture with traditional measurements. The shape defined by these landmarks can then be processed and analysed via statistical methods. We compared the TMJ shapes of wolves and dogs in a principal component analysis and tested the influence of cranial size on shape in a regression. We then tested the covariation with specific skull shape features (rostral proportions and skull flexion) in multiple partial least squares analyses. Moreover, we calculated the amount of TMJ variation explained by these skull shape features, giving information about the degree of integration.

As an important component of the masticatory apparatus, we expect the TMJ to be highly integrated. If, however, the TMJ integration in the skull proves to be limited, meaning that TMJ forms can only be partially explained by skull form or not at all, the TMJ must be regarded as an independent part of the skull, with its final shape determined by region-specific genes or force-induced bone growth. Our results will establish general principles of TMJ formation which might not only account for dogs, but also for mammals in general.

2. Methods

2.1. Sample

Our sample comprised 214 adult dog skulls (both sexes, 129 different pure or mixed breeds). We analysed a wide variety in skull shapes in domestic dogs, including breeds with known peculiarities of the TMJ (Supplementary material Table S1). In addition, we included a geographically diverse sample of 60 wolf skulls (adult, both sexes) originating from European (n = 30), Asian (n = 14) and North American (n = 16) populations to measure the intraspecific TMJ variation of *Canis lupus* in the wild. We took skulls from multiple museums and scanned them with a clinical computed tomography (CT) scanner at various locations (Table S2).

2.2. 3D-reconstruction and landmarks

We volume-rendered the CT scanned skulls in Avizo v. 7 (FEI Visualization Sciences Group) based on a predefined greyscale threshold. In total, we digitized 53 landmarks to capture the external shape of the cranium and the mandible, the mandibular fossa and the condylar process (Fig. 1; Table S3). We placed 29 of these directly on the volume renderings of the skulls (18 on the cranium and 11 on the mandible), and the remaining 24 on 3D surface renderings of the TMJ (12 on the mandibular fossa and 12 on the condylar process) also generated in Avizo v.7 using the same greyscale threshold.

2.3. Geometric morphometric analysis

We performed all analyses in MorphoJ and PAST (Hammer et al., 2001; Klingenberg, 2011). First, we imported the landmark configuration in MorphoJ. We then subdivided it into separate landmark sets for the cranium, the mandibular fossa, the mandible and the condylar process (except for the one-block partial least squares analyses [1B-PLS], for which the cranium and mandibular fossa as well as mandible and condylar process were left undivided). We then Procrustes-fitted these landmark sets to eliminate existing differences in orientation, size and location. This procedure resulted in shape variables (procrustes coordinates), which were used in all subsequent analyses.

- First, we performed a principal component analysis (PCA) to analyse the shape variation of the condylar process and the mandibular fossa in both wolves and domestic dogs.
- By regressing logarithmised centroid size of the cranium on the shapes of the condylar process and mandibular fossa, we tested for TMJ covariation with size.
- To analyse the covariation patterns of TMJ and skull shape, more specifically with skull flexion and relative rostrum length, we performed both one-block and two-block partial least squares analyses (1B- and 2B-PLS). While the 1B-PLS focuses on the relative size and orientation of the structures in question, the 2B-PLS compares both blocks irrespective of these factors. To correct the covariation patterns for allometric effects, we based the PLS analyses on the residuals the regression of cranial size on TMJ shape.
- Finally, as a measure for the integration of the TMJ, we calculated the amount of TMJ variation that can be explained by covariation with the skull. For that purpose, we divided the variance of PLS scores of either mandibular fossa or condylar process by the sum of eigenvalues resulting from their respective PCA. To facilitate the interpretation of shape changes, we created surface models of the cranium, the mandible, the mandibular fossa and the condylar process in Avizo and warped them using 'Landmark' (IDAV, University of California, Davis, USA) based on MorphoJ coordinates.

2.4. Intraobserver error

Computed tomography processing and landmark digitization was performed by a single operator (SC) only. We measured intraobserver error by producing three landmark set replicates for a

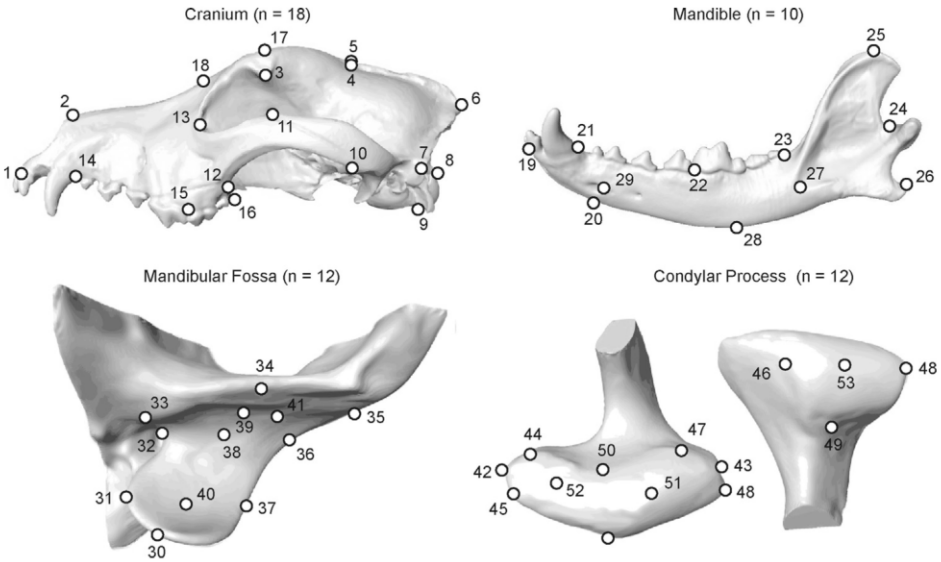

Fig. 1. The landmark set (total n = 53) which was used in our study and which captures the external shape of the cranium and the mandible (lateral perspective), the mandibular fossa (rostral perspective) and the condylar process (dorsal and ventral perspective). A detailed description of every landmark is given in Table S3.

test sample of 15 individuals and tested the variance of replicates as compared to the morphological variance between individuals in a Procrustes ANOVA. The test showed that morphological differences between individuals were highly significant ($p = < 0.0001$; Table S4). The observer's error was slightly higher for the condylar process and mandibular fossa than for the cranium and mandible (cranium 1.5%, mandible 2.8%, mandibular fossa 6.5%, condylar process 8.2%, for details see Table S4). We took this error into consideration by solely interpreting highly significant PLS results that explain most of the covariation and by presenting only the top three principal components (PCs) that explain most of the variation in the dataset.

3. Results

3.1. Variation of the TMJ in wolves and in domestic dog breeds

Fig. 2 shows the top three PCs which account for 47.8% of the total mandibular fossa variation. PC1 (19.4%) reflects variation in the rostro-caudal expansion of the retroarticular process and its orientation. PC2 (17%) is associated with variations in the breadth of the retroarticular process. In PC3 (11.4%), variation is in the medio-lateral expansion of the mandibular fossa. The mean shape of the wolf TMJ can be characterized as medio-laterally wide and fitted with a retroarticular process projecting towards the front (Fig. 2). Relative to this wolf-like condition, some domestic dogs, especially small brachycephalic dogs, enlarge the morphospace towards medio-laterally narrow mandibular fossa shapes (PC3) with a reduced retroarticular process (PC1). Other dogs, however, retain the wolf-like mandibular fossa.

The top three PCs which account for most of the variation in the condylar process make up 45.3% of the total variation (Fig. 3). Here, PC1 (20.3%) is associated with variation of medio-lateral expansion in the condylar process. In PC2 (16.1%), the condylar process varies from cylindrical to slightly concave and dorso-ventrally extended. PC3 (8.9%) reflects variation in the volume of the medial portion of the condylar process. Wolves display a cylindrical condylar process, and many domestic dogs clearly overlap with wolves in this regard. However, some individuals, which could not be grouped in a meaningful way (neither by size, breed, skull shape nor any other characteristic), deviate from this configuration by having rather medio-laterally narrow (PC1, negative values), dorso-ventrally wide, flat and slightly concave condylar processes (PC2, positive values).

Taken together, both wolves and dogs show some variation in their TMJ shapes. Nevertheless, TMJ shapes found in dogs are more diverse than those found in wolves.

3.2. Covariation of TMJ shape and cranial size

The association of cranial size and mandibular fossa as well as condylar process shape is highly significant ($p = < 0.0001$; correlation mandibular fossa: $r = 0.67$, condylar process: $r = 0.49$, Table 1). Wolves and dogs with large crania tend to have concave mandibular fossae together with a large, ventrally projecting and rostrally curved retroarticular process encompassing a cylindrical mandibular caput (Fig. 4). The spectrum of very small crania is only occupied by domestic dogs. These skulls display mandibular fossae with a small, caudally projecting retroarticular process, which can be slightly convex. It articulates with a rostro-caudally flattened mandibular caput with a slightly concave surface (Fig. 4). Irrespective of their consistently large crania, adult wolves, too, display a wide range of condylar shapes, which shows that the effect of size on TMJ shape is limited (Fig. 4). Variation in cranial size explains 7.5% of the total mandibular fossa shape variation and 3.5% of the shape variation of the condylar process (Table 1). These results support our initial hypothesis that the TMJ is more robust in larger specimens than in smaller ones. In smaller dogs, the TMJ is substantially reduced in that it has small condylar and retroarticular processes.

3.3. Covariation of TMJ and skull shape

The covariation of TMJ shape relative to rostrum length and skull flexion is highly significant (p-values can be found in Table 2). However, these two shape features of the skull explain only small amounts of the total variation in the TMJ. Based on the 2B-PLS (irrespective of relative size and orientation), 8.7% of fossa shape and 13% of condylar process shape can be explained by covariation to relative rostrum length (Table 3). Similarly, skull flexion explains 7.9% of the mandibular fossa variation and 11.7% of the condylar process (2B-PLS, Table 3). Thus, both relative rostrum length and

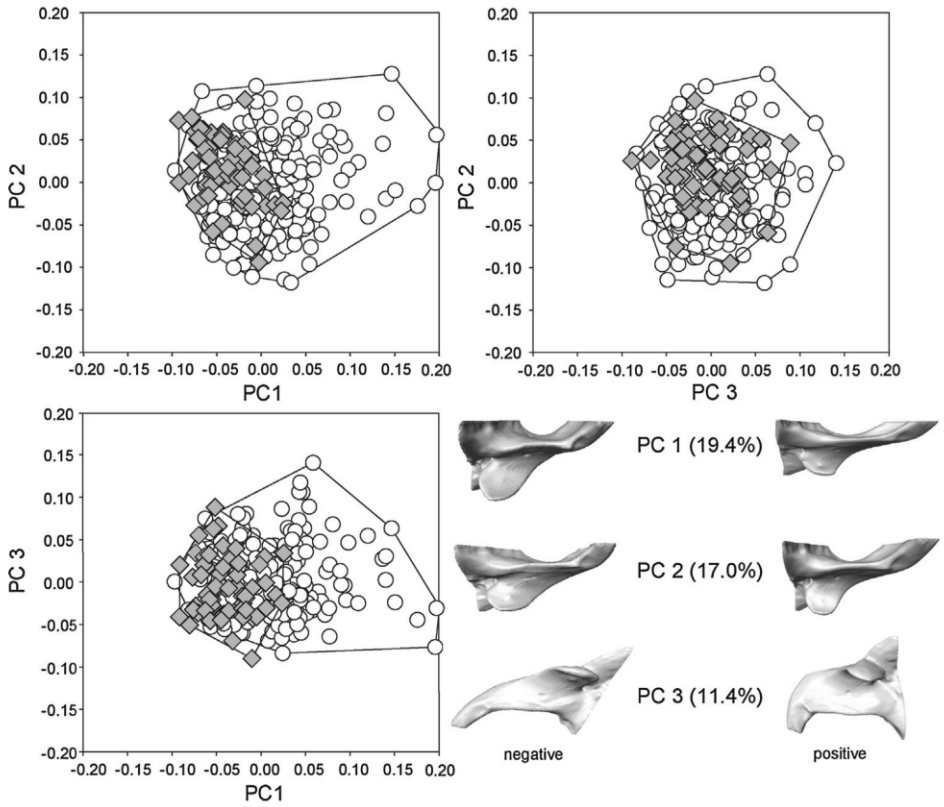

Fig. 2. Principal component analysis plots (PC1–PC3) for the mandibular fossa. The morphospace occupied by wolves is clearly smaller than that of dogs. Grey rectangles – wolves; white circles – dogs.

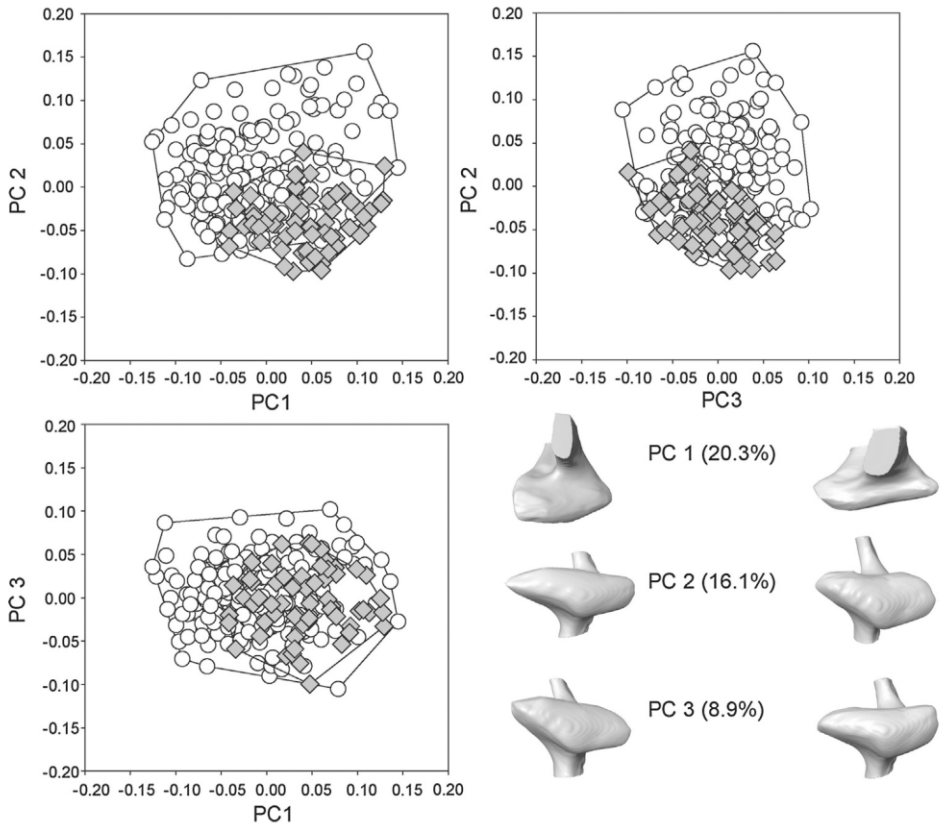

Fig. 3. Principal component analysis plots (PC1–PC3) for the condylar process. The morphospace occupied by wolves is clearly smaller than that of dogs. Grey rectangles – wolves; white circles – dogs.

Table 1
The results of the multivariate regression of the log centroid size of the cranium on the shape of the condylar process and the mandibular fossa.

	Slope	Intercept	r	p	% sums of squares predicted:	p
Condylar process	0.22312	−1.2402	0.48659	0.0001	3.5	<0.0001
Mandibular fossa	0.21576	−1.1992	0.67487	0.0001	7.5	<0.0001

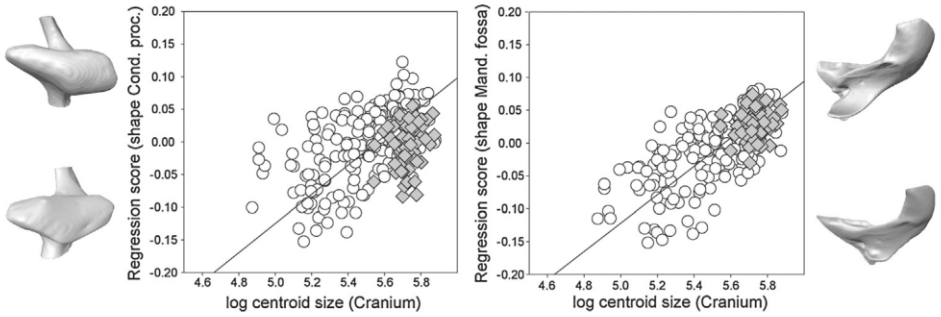

Fig. 4. The allometric component of TMJ shape variation. Warped surfaces of both the condylar process and the mandibular fossa show the shape changes associated with size. Grey rectangles – wolves; white circles – dogs.

Table 2
The results of the 1-Block and 2-Block partial least squares analyses for the covariation of the cranium and the mandibular fossa and the mandible and the condylar process.

			% total covar.	p	r	p	RV	p
Cranium–mandibular fossa	1-Block	PLS 1	91.9	<0.0001	0.94	<0.0001	0.84	<0.0001
		PLS 2	6.4	<0.0001	0.91	<0.0001	0.84	<0.0001
	2-Block	PLS 1	85.1	<0.001	0.59	<0.0001	0.12	<0.0001
		PLS 2	4.5	0.0063	0.39	0.0001	0.12	<0.0001
Mandible– condylar process	1-Block	PLS 1	86.4	<0.001	0.91	<0.001	0.65	<0.001
		PLS 2	7.8	<0.001	0.78	0.096	0.65	<0.001
	2-Block	PLS 1	74.9	<0.0001	0.58	<0.0001	0.15	<0.0001
		PLS 2	13.9	<0.0001	0.40	<0.0001	0.15	<0.0001

Table 3
The percentage of the variation as explained by the covariation of the cranium and the mandibular fossa and the mandible and the condylar process (2B-PLS).

	Sum of eigenvalues (PCA)	Covariation with		% of covar.	Variance of PLS scores	% of variation explained by covariatio
Mandibular fossa	0.0132	Cranium	PLS 1	85.1	0.0012	8.7
	0.0132		PLS 2	4.5	0.0010	7.9
Condylar process	0.0158	Mandible	PLS 1	74.9	0.0021	13.0
	0.0158		PLS 2	13.9	0.0019	11.7

skull flexion leave a considerable amount of TMJ variation unexplained.

The detailed covariation patterns are as follows: regarding TMJ and relative rostrum length covariation, the wolf morphospace spreads over only a narrow range of the covariation spectrum (from mesati- to dolichocephalic), while the range in domestic dogs expands especially towards brachycephalic skulls. In the 1B-PLS, the medio-lateral axis of TMJs is orthogonal to the long axis of the skull in dolichocephalic skulls, whereas, in brachycephalic skulls, it is caudally rotated at its lateral point (1B-PLS 1, Fig. 5A, C). Furthermore, in brachycephalic skulls, the mandibular caput is rostro-caudally flattened and medio-laterally narrowed and articulates with a mandibular fossa that is flat with a low retroarticular process (2B-PLS, Fig. 5B, D). In dolichocephalic skulls and the skulls of wolves, the mandibular caput is cylindrical and medio-laterally widened and articulates with a mandibular fossa that bears a strong, ventrally extended and rostrally curved retroarticular process (2B-PLS, Fig. 5D). This supports our hypothesis that longer rostra covary with robust TMJs.

Regarding the covariation of the TMJ and skull flexion, wolves occupy a range in the covariation spectrum which spreads from airorhynch (dorsally tilted rostrum and mandibular corpus) to orthognath (straight rostrum and mandibular corpus) (Fig. 6). The domestic dogs enlarge the covariation range towards klinorhynchy (ventrally tilted rostrum and mandibular corpus) (Fig. 6A–D). Airorhynch skulls covary with horizontally oriented and medio-laterally narrow mandibular fossae, of which the medio-lateral axis of the TMJ is orthogonal or rostrally rotated to the long axis of the skull (2B-PLS 2, Fig. 6A, B). The condylar process is convex, rostro-caudally extended and ends caudal to the coronoid process (1B-PLS 2, Fig. 6C, D). Klinorhynch skulls covary with caudally inclined and medio-laterally extended mandibular fossae (1B-PLS 2, Fig. 6A). In this case, the medio-lateral axis of the TMJ is orthogonal or caudally rotated to the long axis of the skull (2B-PLS 2, Fig. 6B). The condylar process is rostro-caudally compressed and ends approximately at the height of the coronoid process (1B-PLS 2, Fig. 6C). The mandibular fossa is slightly convex (2B-PLS 2, Fig. 6B) and, accordingly, the articular surface of the condylar process shows a concavity on the medial side resulting in a wave-like shape (2B-PLS 2, Fig. 6D). The hypothesis that skull flexion affects the robustness of the TMJ is not supported by these results.

4. Discussion

Here we investigated the integration of the TMJ in the skulls of wolves and domestic dogs by testing the predictability of TMJ

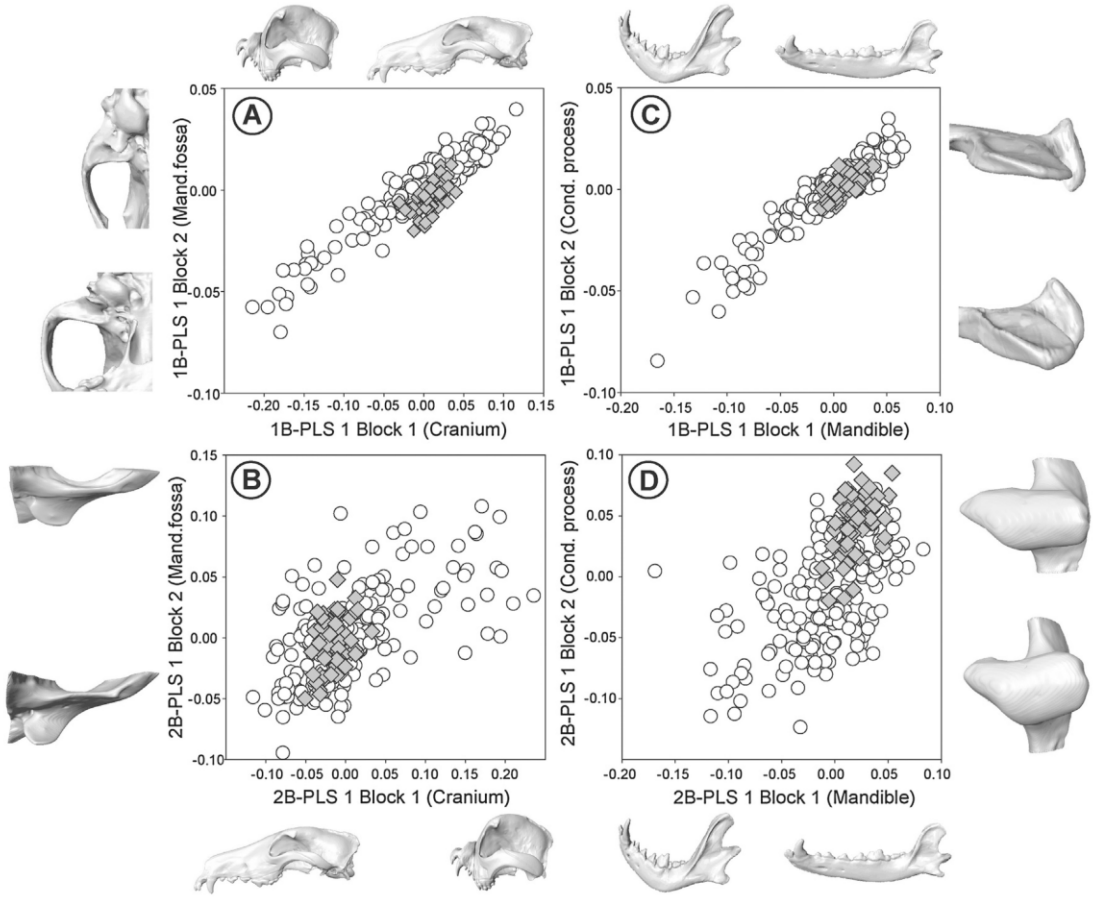

Fig. 5. PLS 1 of the 1B-PLS (A and C) and 2B-PLS (B and D) showing the covariation of cranium and mandibular fossa (A and B) as well as the mandible and the condylar process (C and D) with varying rostral proportions, i.e. dolichocephaly vs. brachycephaly (corrected for allometric effects). Grey rectangles – wolves; white circles – dogs.

shapes based on skull size and shape. We hypothesised that an increase in skull size, dolichocephaly and klinorhynchy would be correlated with a more robust TMJ. Only cranial centroid size and dolichocephaly are clearly associated with more robust TMJs, while klinorhynchy primarily affects the length of the condylar process, the medio-lateral bone distribution at the TMJ and its orientation. More importantly, the amount of TMJ variation explained by skull form variations is limited, pointing towards at least partial structural and perhaps developmental independence of the TMJ.

Although different wolf subspecies from Asia, Europe and North America were included in our sample, the TMJ in wolves is remarkably consistent when compared to the TMJ in domestic dogs (Figs. 2 and 3). It seems that natural selection of masticatory function and carnassial alignment restricted variation in the wolf, resulting in a relatively homogenous hinge-like morphology, which guarantees bite precision, stability and safety from disarticulation (Scapino, 1965; Dessem and Druzinsky, 1992; Crompton et al., 2006). In modern day dog breeds, especially those bred mainly for companionship, functionality and stability of the TMJ regarding prey capture and highly demanded carnassial function have become obsolete. Dog food industries and pet breeding cultures alleviated the selective pressure on a functional masticatory apparatus in dogs, leading to the increased diversity, not only in overall skull shape (Drake and Klingenberg, 2010) but in TMJ shape as well (Figs. 2, 3). At an early stage of domestication, the alliance with humans increased hunting efficiency (Clutton-Brock, 1995) and thereby decreased selective pressures for dogs, opening new opportunities for their skulls to vary. Today, these variations allow for the differentiation of early dogs from wolves (Clutton-Brock, 1995; Drake et al., 2015). With the shift from a hunter-gatherer to an agriculture-based lifestyle in humans, dogs increasingly relied on starch rich food sources (Axelsson et al. 2013). This likely allowed for the loss of even more biomechanical constraints in the skulls of dogs as well as humans (von Cramon-Taubadel, 2011). To this day, human care allows for skull variations in the dog that under natural conditions would significantly reduce evolutionary fitness. Even pathological traits, such as short muzzles that result in breathing problems and malocclusions, are artificially preserved (Colyer, 1990; Koch et al., 2003). In the TMJ of some dogs, the transition from a foraging to a human-sustained lifestyle enabled the reduction of the retroarticular process on the mandibular fossa as an important stabilizing structure and a medio-lateral narrowing of the TMJ (Fig. 2). The condylar process became more rostrocaudally flattened or even slightly concave in appearance, although most dog breeds retained the wolf-like cylindrical form (Fig. 3). Villamizar-Martinez et al. (2016), who investigated the TMJ variation in a sample of 48 dogs from eight different breeds via linear measurements and angles, found similar types of variation and aimed at establishing a morphology-based TMJ classification. In the breeds they investigated, TMJs ranged from forms with irregularly formed condylar processes, shallow mandibular fossae and reduced retroarticular processes, which they defined as type C, to forms with more uniformly shaped condylar processes, prominent retroarticular processes and deeper mandibular fossae, which they defined as type A; type B was defined as somewhere between these two extremes (Villamizar-Martinez et al., 2016). We would oppose

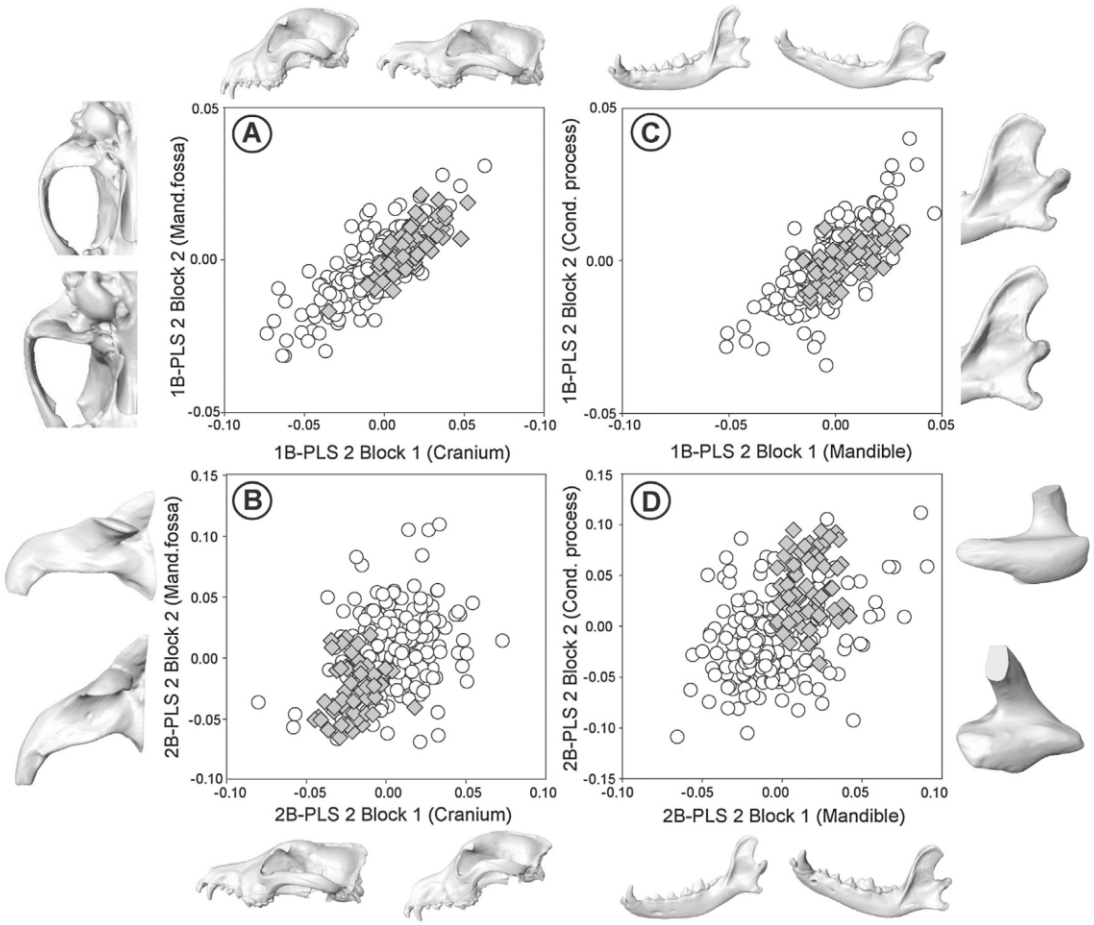

Fig. 6. PLS 2 of the 1B-PLS (A and C) and 2B-PLS (B and D) showing the covariation of cranium and mandibular fossa (A and B) and of the mandible and the condylar process (C and D) with varying skull flexion (corrected for allometric effects). Grey rectangles – wolves; white circles – dogs.

this kind of classification because our analysis shows that the variation of the dog TMJ does not form any distinct groups, but rather, changes gradually from one configuration to another in every direction of variation, making classifications like these highly arbitrary.

Especially with TMJ shapes displaying only shallow mandibular fossae and reduced retroarticular processes, some domestic dog breeds increasingly diverged from typical carnivorous, hinge-like TMJ morphologies towards sledge-like shapes that are typical of omnivorous or herbivorous mammals. Primates, artiodactyls and rodents, for instance, have almost spherical condylar processes that articulate with mandibular fossae displaying (if present) only shallow bony projections for stability, allowing complex three-dimensional chewing movements for the consumption of fibrous plant material (Herring, 1976; Byrd, 1981; Menegaz et al., 2015). The TMJs of mustelids in a study by Dumont et al. (2016) showed a similar gradient of variation as found in dogs and wolves, which in their study was associated with differing feeding ecology. While carnivorous mustelids showed deep, medio-laterally wide, rostro-caudally narrow mandibular fossae with strong processes, herbivorous species had weaker processes and the mandibular fossae were rostro-caudally widened and more ventrally oriented. Further, they proposed that these differences in shape might be associated with differently oriented joint reaction forces and varying ranges of motion during mastication. Analysis of the masticatory kinematics of differently shaped TMJs in both dogs and mustelids could thus lead to a deeper understanding of the functional morphology of the TMJ.

Apart from this general trend to more unstable TMJ shapes in some dog breeds, we found a highly significant correlation between skull size and degree of TMJ robustness in domestic dogs and wolves (Table 1, Fig. 4). Structures of the head related to masticatory function (e.g. temporal muscle area) scale either isometrically (Penrose et al., 2016) or in a positively allometric manner relative to body size (Radinsky, 1981). In the mandibular fossa, size affects the projection of the retroarticular process: it is very shallow in small-skulled dogs and very pronounced in wolves and large-skulled dogs. In the condylar process, large cranial centroid size is associated with a cylindrical and convex shape, while small condylar processes are associated with flat shapes (Fig. 4). This is in support of a study by Villamizar-Martinez et al. (2016), which found similar TMJ shapes to be associated with body size. Although general covariation patterns exist, considerable variation can still be found in both large and small dogs. Therefore, size alone does not explain the existent variation. When the integration of skull shape and TMJ shape was tested, we expected to find more robust TMJs in long-muzzled and klinorhynch individuals. Our results, however, only partially supported this hypothesis: while relative rostrum length was correlated with TMJ robustness, skull flexion was not. Dolichocephalic skulls covary with more robust, well-formed TMJs, while brachycephaly is associated with rather gracile and reduced TMJ features. When TMJ orientation is considered (1B-PLS), brachycephalic dogs have TMJs whose medio-lateral axis is rather oblique to the medio-lateral axis of the skull, while dolichocephalics and wolves, in contrast, have TMJs with the medio-lateral axis in congruence with

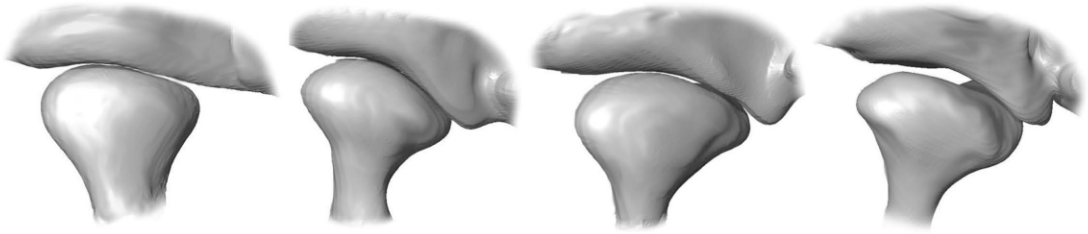

Fig. 7. TMJ variation in four adult French Bulldogs: relatively uniform skull shapes in one dog breed can show a diverse range of TMJ shapes, implying a certain degree of modular independence of the TMJ.

the medio-lateral axis of the skull (1B-PLS Fig. 5). Thus, the TMJ axis rotation may be a result of reduced space availability in short-faced skulls. Our results for skull flexion indicated no relation to gracility or robustness. While klinorhynch skulls covary with a short condylar process and a caudally inclined, medio-laterally wide mandibular fossa, airorhynch skulls covary with a long condylar process and a horizontally oriented, medio-laterally narrow mandibular fossa (1B-PLS, PLS 2; Fig. 6). Villamizar-Martinez et al. (2016), in their study on TMJ variation in dogs, found no relation of TMJ shape and skull configuration (brachycephalic or mesaticephalic; dolichocephalics were not included). Our study shows that correlations of skull and TMJ shape clearly exist, although there are numerous exceptions to the described pattern.

In dogs, bone-morphogenetic protein 3 expression (BMP3) has been demonstrated to be linked to both facial length (Schoenebeck et al., 2012) and bone formation at the TMJ (Aspenberg et al., 2000). Thus, the reduced appearance of the TMJ in brachycephalic dogs is likely a by-product of generally reduced bone formation in the skull. Similarly, insulin-like-growth factors have previously been linked to both body size regulation in dogs (Rimbault et al., 2013) and growth of the TMJ (Delatte et al., 2004). When comparing our results for dogs and wolves to other studies on TMJ integration, we found striking similarities to the covariation patterns in platyrrhine primates, suggesting similar underlying morphogenetic factors. In these primates, small, short-faced skulls covary with rostro-caudally long and medio-laterally narrow articular surfaces, shallow retroarticular processes and a relatively large, but flat condylar process (Terhune et al., 2015; Terhune, 2013b). Long-faced and large skulls covary with a rostro-caudally short, but medio-laterally widened articular surface, concave mandibular fossae, a prominent retroarticular process and a relatively small condylar process compared to skull length (Terhune, 2013b; Terhune et al., 2015). Quantitative trait loci, which regulate bone growth in the skull (Chase et al., 2002), could explain these similar covariation patterns present in both primates and dogs which result in more robust or gracile TMJ features with more robust or gracile skeletal features, respectively.

In dogs and wolves, the covariation of skull geometry and TMJ shape explains only a small percentage of the variation in the TMJ despite being highly significant. For instance, Vollmerhaus and Roos (1996) described the specific concave shape of the condylar process in dachshunds and the convex, knob-like retroarticular process as its counterpart on the cranium, a feature which is typical for this breed, but seemingly unrelated to other features of the skull (Vollmerhaus and Roos, 1996). Barrette (1986) described a congenital, highly aberrant alteration of both TMJs in a wolf in which, similar to the dachshund example, the rest of the skull was not affected in conjunction with the aberration (Barrette, 1986). Moreover, dogs of the same breed with similar skull shapes can have very differently shaped TMJs (Dickie and Sullivan, 2001; Dickie et al., 2002). In our sample, this was especially striking in French Bulldogs, in which the TMJs are very differently shaped (Fig. 7). The variation in TMJ shape in these specimens could not be accounted for by sex or age differences. This again proves that TMJ variation is not necessarily related to variations in skull geometry. Since the skulls of these dogs are more or less similarly shaped, TMJ variations like these are likely to arise from localised effects such as force-induced bone growth (Humphreys, 1932; Moffett, 1966; Beek et al., 2000; Herring and Liu, 2001; Herring et al., 2002; Ravosa et al., 2007; Tanaka et al., 2008; Von den Hoff and Delatte, 2008). The vascular endothelial growth factor, the insulin like growth factor and the fibroblast growth factor, for example, have been described to be highly responsive to mechanical stimuli at the TMJ (Fuentes et al., 2002; Von den Hoff and Delatte, 2008). This conclusion, however, would require further experimental testing. If true, the final shape of the joint could be a result of dental malocclusions and, consequently, altered chewing movements that partially distort the underlying genetic determination of the joint's shape.

In closing, we would like to state some limitations of this study. Studies in geometric morphometrics require high-quality landmark sets to produce meaningful results (Arnqvist and Martensson, 1998), but finding homologous landmarks is difficult in a structure like the TMJ, which offers only round outlines and unstructured surfaces. In this study, we relied mostly on landmarks which describe local maxima and minima of a structure, such as the tip of the retroarticular process (known as type II landmarks; Zelditch et al., 2012). Based on these, more landmarks could be defined on otherwise unstructured parts of the joint, such as the half-distance between the medial and lateral margins of the mandibular fossa. Otherwise important information, such as the convexity or concavity of the articular surface, would have been lost. This of course introduces some uncertainties about landmark homology. Thus, we performed an ANOVA which compares the variation between individuals and the variation between replicates (Klingenberg and McIntyre, 1998). The results showed that, although uncertainties in landmark placement introduce some error, the variation between the individuals is large enough to produce meaningful results (Fruciano, 2016). Nonetheless, conclusions must always be drawn with caution, since especially quantitative results in studies using geometric morphometrics can vary depending on sample size (Cardini and Elton, 2007), landmark number and distribution.

5. Conclusion

The domestication of the wolf and artificial selection of the dog resulted in a great variation of skull and TMJ shapes. Because of human husbandry, some dog breeds bear TMJs which appear less stable and load-resistant than those of wolves and long-snouted dogs. Although TMJ shape variation explained by covariation with the surrounding skull is relatively low, relative rostrum length, skull flexion and size are directly connected to specific features of the TMJ. The patterns found in our dog and wolf sample resemble those found in some primates. Yet, dogs of the same breed with quite similar skull shapes can exhibit highly variable TMJ shapes. This implies a low integration of the joint in the skull. It is possible that genes and developmental processes, specifically those affecting the TMJ

area, lead to a certain degree of structural independence of the TMJ. Similar factors are likely to predominate during the development of new TMJ morphologies, not only in dogs, but generally in the course of mammalian evolution.

Author contributions

SC, MSF and KK conceived the study. SC collected the data, performed the analyses and drafted the manuscript, which was afterwards critically revised with MSF and KK. All authors approved the article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aanat.2017.08.003.

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3 SYNOPSIS

The mammalian masticatory apparatus fulfils a wide range of functions. In spite of its name, the teeth, the skull, the jaw joints, the muscles and the tongue interact not only to masticate food but also to capture prey, to handle offspring or food items, to fight competitors and predators alike, to groom the body or to communicate both inter- and intraspecifically. This imposes different selection pressures on the head and skull, which has to cope with this diversity of functions equally well in order to maximize evolutionary fitness. In the wolf, this diversity of selection pressures limits skull variation in the wild. In the domestic dog however, human husbandry has largely alleviated natural selective pressures. Instead, breeders have artificially selected beneficial traits in domestic dogs for a diverse range of tasks from herding and retrieving to fighting and hunting. Especially since the Victorian era fancy phenotypes were the focus of selection (Freedman et al., 2016), resulting in a wide range of skull forms with increasing distinctiveness (Drake and Klingenberg, 2008, 2010). Disproportions, such as between the cranium and the mandible in bulldogs (Colyer, 1990) and localized malformations, such as the deformed TMJs in dachshunds (Vollmerhaus and Roos, 1996), constitute a part of this variation. This led to the hypothesis that a reduced integration strength among the skull modules in dogs and changes in their integration pattern when compared to wolves might have played an important role during the diversification of the dog skull. Thus, the goal of this doctoral thesis was to investigate the balance of modularity and integration in feral to domesticated *Canis lupus* that might explain the enormous skull shape disparity in domestic dogs.

Study I: The integration strength and pattern in domestic dog and wolf skulls

In a first study (presented in chapter 2.1) it was tested, whether domestic dogs have less integrated skulls than wolves and whether the pattern of covariation of the skull and dentition and of the internal modules of the cranium and mandible has been altered by artificial selection. The results unexpectedly indicated a high integration strength in domestic dog skulls, which stood in no relation to the larger disparity of the cranium and the mandible (both, when dental landmarks were considered or neglected). Even the tooth rows were highly integrated with the mandible and the cranium with their shape mainly determined by the surrounding skull, although previous studies implied only limited integration of the skull and the dentition (Dayan et al., 2002; Cobourne and Sharpe, 2003; Boughner and Hallgrímsson, 2008; Gómez-Robles and Polly, 2012; Le Cabec et al., 2012; Asahara, 2013). Also the pattern of integration of skull modules was unchanged in

domestic dogs when compared to the wolf. This conservation of ancestral (wolf-like) covariation patterns in domestic dogs was demonstrated by extrapolating covariation trajectories which were based solely on wolf data. By this procedure, dog-like (e.g. brachycephlic and klinorhynch) skull shapes with dog-like covariation patterns among the skull modules were generated. Yet, while in the domestic dog the skull shape variation from brachycephalic to dolichocephalic dominated the covariation pattern, for wolves, varying skull flexion and robustness were dominant. This indicates that humans concentrated their selection efforts on other, possibly more easily modifiable traits than on those which create most of the variation in the wild.

Study II: The predictability of TMJ shapes from skull geometry of domestic dogs and wolves

Stimulated by these surprising results, a second study was conceived, which was presented in chapter 2.2. When integration is prevalent in the skulls of domestic dogs, also the shape of specific and relatively small parts of it should be predictable on the basis of the geometry of the whole skull. Thus, in this study it was investigated whether the high level of integration also applies to the temporomandibular joint (TMJ) of domestic dogs and wolves and whether the variation of TMJ shape is a mere by-product of the large skull shape disparity. More precisely, it was hypothesized that increased skeletal growth which results in larger, dolichocephalic and klinorhynch skulls will also result in more robust TMJs with a large retroarticular process and a voluminous condyle as opposed to TMJs with a slender condyle, lacking a retroarticular process in small, brachycephalic and airorhynch skulls. It was found that some features of the TMJ can be explained by the integration with the surrounding skull (increased robustness with dolichocephaly and size). Again, as in the first study, the variation of the skull from brachycephalic to dolichocephalic was the direction of variation that determined most of the covariation pattern. However, some of the TMJ variation found cannot be explained by “global factors” which affect both the skull in general and the TMJ in specific (Mitteroecker and Bookstein, 2008). Especially the occurrence of differently shaped TMJs in very similar skulls (such as in French Bulldogs) implies that this variation is due to local factors (e.g. localized acting genes or the interaction with surrounding soft tissue). Thus, this study shows the potential of a functionally integrated structure like the TMJ to develop new shapes once selective pressures on functionality are reduced. Beside these observations, this study was the first to measure and compare the disparity of TMJ shapes in dogs and wolves, which was noted before but never comprehensively investigated (Stewart et al., 1975; Gibbs, 1977; Robins and Grandage, 1977; Johnson, 1979; Hoppe and Svalastoga, 1980; Ström et al.,

1988; Vollmerhaus and Roos, 1996; Dickie and Sullivan, 2001; Dickie et al., 2002; Macready et al., 2010; Schwarz et al., 2002; Lerer et al., 2014; Villamizar-Martinez et al., 2016).

Diversification under high integration

When bringing the results of both studies together, both show that few types of variation (variations in relative rostral length, skull flexion, size and varying robustness) explain most of the covariation pattern among skull modules in *Canis lupus*. This applies to the internal modules of the mandible and the cranium, and the tooth rows in the skull, as much as to the TMJ. These uniform patterns signal high integration of the skull. In the dog, the variation from brachycephalic to dolichocephalic determined most of the covariation pattern, followed by varying skull flexion and robustness. In the wolf, varying degrees of skull flexion and robustness were dominant, but varying relative rostrum length also guided the covariation of their skull modules. That similar types of variation occurred repeatedly and determine the covariation pattern throughout separate analyses and also when based on different data sets shows that the variation must be caused to a large part by “global factors” that affect wide parts of the skull (Mitteroecker and Bookstein, 2008). The results presented here closely resemble the conclusion drawn for another domestic animal, the pigeon, which also shows mostly integrated variation (Young et al., 2017). Notably, the variation of the dog and wolf skull was continuous for every type of variation (with regard to relative rostrum length, skull flexion and robustness), without the formation of distinct groups. The historical segregation of dogs into individual groups on the basis of their skull morphology (brachycephalic, mesaticephalic, dolichocephalic) is thus highly arbitrary (similarly noted by Georgevsky et al. (2014)), which is why terms like “brachycephalic” or “dolichocephalic” should rather be understood as extremes along a continuous scale and not as a distinct groups of individuals. Typically, traits that vary by degree like these are of multifactorial origin and controlled by the environment, genetics and epigenetics (Emery et al., 2012; Charmantier et al., 2014). Moreover, they are not inherited according to simple dominant or recessive patterns. Instead, they are controlled by a polygenic set on different gene loci that work additive or quantitative resulting in traits with normal distribution (Emery et al., 2012; Charmantier et al., 2014). In the face of the continuous variation of skull characteristics, these so called quantitative trait loci (QTL) lend an explanation for the observed skull shape variation of dogs (Fondon and Garner, 2004; Wu et al., 2004; Fondon and Garner, 2007). As a study by Chase et al. (2002) showed, this variation is not restricted to the skull but responsible for highly integrated trait characteristics throughout the whole body of domestic dogs. Influenced by natural

selection, the shapes of the cranium and the mandible (including tooth row shapes and TMJ shapes) of wolves are restricted by functionality and thus reach only a low disparity level and occupy only small areas of the morphospace in all analyses. Dogs, however, could overcome functional constraints through human care, which is reflected in the analyses by higher disparity values and larger morphospace occupation (shown in Study II). Yet, the radiation followed the ancestral (wolf-like) covariation trajectories, which adheres to ancestral developmental constraints, and thus produced no real novelties (Hallgrímsson et al., 2012). Again, similar as in domestic pigeons (Young et al. 2017) dogs recapitulate the principal directions of variation of their non-domesticated (and thus ancestral) form but surpass the natural shape disparity manifold, probably caused by increased or decreased gene expression on QTL (Hallgrímsson et al., 2012) as a "global factor" (Mitteroecker and Bookstein, 2008).

Obviously, a large amount of variation in the domestic dog arises not from the quasi-independent development of skull modules, but through the integrated variation of the whole skull as one unit. This shows that high integration does not limit the formation of new skull shapes with high disparity levels, rather the integration of skull modules drives the main variation along few main trajectories of variation (Goswami and Polly, 2010; Young et al., 2017). A modular dissociation of traits is no necessity to create an abundance of shapes. As Schluter (1996) already noted, adaptive change is often constrained to few dimensions, especially when traits are genetically correlated. This drives diversification predominantly along "lines of least resistance" (Schluter, 1996). The selection along these integrated trajectories might be easier than selection going across these trajectories and Fondon and Garner (2004) and Drake and Klingenberg (2008) gave examples for increasing degree of severity of traits along those lines.

Shared patterns of variation and deep-time conservation of underlying developmental factors in amniotes

The main types of variation and covariation in the dog skull do not only resemble those of wolves, they surprisingly even parallel the main dimensions of variation in other animal groups implying the deep-time conservation of underlying genetic and developmental factors (such as genetic or developmental programs). Several other studies also came to this main conclusion and this thesis corroborates these results (Goswami, 2006a, 2006b; Mitteroecker and Bookstein, 2008; Marroig et al., 2009; Porto et al., 2009; Drake and Klingenberg, 2010; Singh et al., 2012; Figueirido et al., 2013; Goswami et al., 2014; Young et al., 2017).

Most of the variation in the dog skull and the covariation of its modules was explained by varying relative rostrum length. A similar type of variation is known for humans, bats, great apes, antelopes, rodents, mongooses, carnivores and marsupials (Wroe and Milne, 2007; Hautier et al., 2012; Singh et al., 2012; Cardini and Polly, 2013; Figueirido et al., 2013; Cardini et al., 2015a). Especially the so called CREA-pattern (Craniofacial Evolutionary Allometry), which describes that small animals have shorter faces and paedomorphic traits while larger animals have longer faces, can probably be regarded as a general rule that is responsible for much of the skull disparity among amniotes and mammals in particular (Cardini and Polly, 2013; Cardini et al., 2015a; Tamagnini et al., 2017). Since bone-morphogenetic proteins and the activity of neural crest cells populating the developing face have repeatedly been associated with facial length (Abzhanov et al., 2004; Wu et al., 2004; Helms and Brugmann, 2007; Schoenebeck et al., 2012; Wilkins et al., 2014), these factors are likely candidates to contribute to this type of variation. Similarly, also the variation of the dog skull with regard to flexion and robustness resembles patterns of variation in other mammals (Radinsky, 1981; Sears et al., 2007; Hautier et al., 2012; Asahara, 2013; Figueirido et al., 2013). Klinorhynchy was related before to the runt related transcription factor 2 (Fondon and Garner, 2004, 2007; Sears et al., 2007), robustness and size (Rimbault et al., 2013) were connected to each other by differential scaling of the brain and the muscles (Radinsky, 1981; Penrose et al., 2016).

It seems evident, that studies with domestic animals can show the maximal potential of ancestral patterns of variation on an intraspecific level (Drake and Klingenberg, 2010; Young et al., 2017). Some of these domestic forms resemble wild species as has been shown impressively especially for pigeons (Young et al., 2017) and dogs (Drake and Klingenberg, 2010). Because of this finding, it is likely that both intraspecific and interspecific variation of the skull shape are two sides of the same coin (Helms and Brugmann, 2007). In the wild however, natural selective pressures and functional constraints restrict possible variations in the morphospace. Some forms occur only in human directed natural experiment of domestic species, but do not prevail under natural conditions. This can be observed in young populations of feral dogs, which face high rates of juvenile mortality, which are still dependent on the (unintentional) food provision by humans, and are thus not self-sustaining (Boitani and Ciucci, 1995). Long term survival of such populations seems only possible by returning to “wild type” features such as longer snouts as it is known from the Australian dingo (Crowther et al., 2014).

Disproportions and malformations in highly integrated skulls

The inspiration for this thesis arose from frequently occurring disproportions and locally restricted malformations in the domestic dog skull. These are for example the oversized teeth in small or brachycephalic dogs resulting in tooth crowding (McKeown, 1975; Colyer, 1990), severely overshot or undershot bites (Grüneberg and Lea, 1940; Colyer, 1990) or variations of the TMJ which were seemingly unrelated to the morphology of the rest of the skull (Vollmerhaus and Roos, 1996). Yet, since both studies point to a still prevalent strong integration of the skulls of domestic dogs, how do these phenomena, which imply a quasi-independence of traits, integrate in these results?

The high integration in the domestic dog skull and some observable disproportions are, counter-intuitively, not in conflict. Although disproportions between the teeth and the jaw and between the mandible and the cranium can occur, they occur in predictable ways. A specific shape in one module is mostly correlated with a specific shape in the other module. If the quasi-independence of single modules would be dominant, predictions on covarying shapes could not be made. When taking the example of disproportions between the mandible and the cranium, our study shows that the mandible has a lower tendency to vary resulting in lower disparity values, while the cranium shows higher plasticity, especially with regard to rostro-caudally restricted or amplified growth, resulting in higher disparity values. Drake et al. (2017) could recently show using a sample of fossil dog skulls, that mandibles do not evolve as fast as crania. Also for modern dog breeds this would imply that mandibles are more stable in their morphology and less affected by breeding efforts, while the cranium has a higher tendency for morphological change. The reasons for this are not known, but the patterns of suture closure in the cranium might be a possible explanation (Geiger and Haussman, 2016). In regard to disproportions between the cranium and the mandible, this means that a reduced growth of the rostrum is only partly followed by the mandible, resulting in undershot bites in brachycephalics (Colyer, 1990). Also on the opposite end of the spectrum, increased growth of the rostrum leading to dolichocephalic crania is frequently not in congruence with the mandible resulting in overshot bites (Colyer, 1990). Similarly, the covariation of the rostrum and the braincase in the cranium, of the ramus and corpus of the mandible, between tooth rows and skull and mostly also between TMJ and skull happens in predictable ways although disproportions imply a reduced integration. Remarkably, by extrapolating the PLS covariation trajectories of wolves (as done in Study I), skull shapes which are very similar to those of dog breeds with known disproportions (e.g. brachycephalics with crowded und oversized teeth) were generated. This shows that these disproportions are not the result

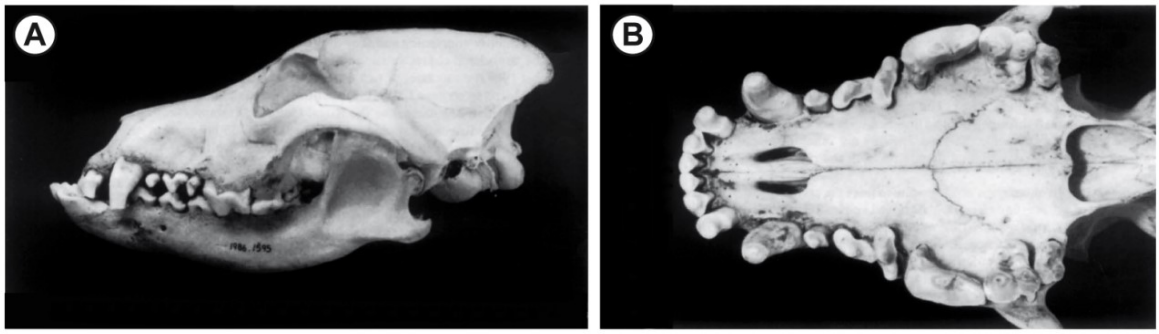

Fig. 2 Photos of the skull of a male wolf (*Canis lupus arctos*, BM(NH) 1986.1595) first published in Clutton-Brock et al. (1994) with A) undershot jaw from lateral view and B) crowded teeth from palatal view, showing that both phenomena also occur in wild wolves. Reproduced by permission of John Wiley and Sons, modified. Permission to reuse must be obtained from the rightsholder.

of altered covariation patterns through artificial selection. The potential to develop similar disproportions is already existent in the covariation patterns of wolves (Fig. 2), but mostly kept in check by natural selective pressures and biomechanical constraints. Through human husbandry new growth and remodelling playgrounds are opened within the genetically guided limits derived on the phylogenetic preconditions. The conservation of main covariation patterns in the domestic dog is however not self-evident. At least there would have been the possibility for human breeders to aim at skull shapes which break wild-type covariation patterns. However, constraints given by the genetic architecture and developmental mechanisms might restrict possibilities to cross these boundaries. So the “embryonic architecture in amniotes“ is likely to determine the limitations which functional phenotypes can be produced and which not (Young et al., 2017).

Beside these disproportions which can be explained by the main covariation patterns among skull modules in domestic dogs and wolves there are localized malformations which obviously diverge from these patterns. In Study II especially the TMJs of French bulldogs and other brachycephalics showed a diverse range of shapes in spite of the relatively uniform shape of their skull (Dickie and Sullivan, 2001; Dickie et al., 2002). Here, on lower hierarchical levels and in smaller structures, it seems that local factors such as force induced bone growth have a greater impact and thus increase the detectable quasi-independence of traits (Humphreys, 1932; Moffett, 1966; Beek et al., 2000; Herring and Liu, 2001; Herring et al., 2002; Ravosa et al., 2007; Tanaka et al., 2008; Von den Hoff and Delatte, 2008). Moreover, maloccluding teeth could force the jaw to chewing movements which ultimately alter the shape of the TMJ since the possible impact of occlusion on the integration of the masticatory system has been highlighted before (Polly, 2012; Smits and Evans, 2012). Yet, also these very localized malformations do also occur in wild wolves

(Barrette, 1986), although more rarely described in the literature and are thus no novelties that arose from artificial selection.

Materials, methods and limitations

Finally, some reasons for the choice of methods shall be given and their limitations shall be discussed. For both studies, skulls of adult domestic dogs and wolves of both sexes were carefully chosen from various museum collections. The sample was assembled to reflect the actual disparity of skull shapes in domestic dogs, from brachycephalic to dolichocephalic, from airorhynch to klinorhynch, from small to large and from robust to gracile. Also the wolf skulls were chosen to represent a range of variation comparable to that of wild populations which is why specimens originating from Asia, Europe and North America have been included (for further details see supplementary material of both studies in the appendix). Similar types of variation in the sample of the given analyses and those of other studies (e.g. Drake and Klingenberg, 2010), which in some cases included larger samples, provided confidence, that the actual diversity of shapes was successfully approximated.

The skulls were scanned with a clinical computed tomography scanner and the landmarks were digitized on digitally 3D-reconstructed (volume rendered) skulls using Avizo v. 7 (FEI Visualization Sciences Group) (For details concerning the computed tomography scans and the landmarks chosen please refer to the supplementary material of Study I and II in the appendix). Two major alternatives to this procedure exist: first, the use of digital photographs and the virtual digitization of landmarks (Meloro et al., 2017) and second, the digitization of landmarks using a MicroScribe digitizer (Singh et al., 2012). For this study, computed tomography scans and virtual 3D reconstructions were used in spite of higher costs and larger effort since 1) CT scans allow the study of internal structures without harming the specimen. Using a MicroScribe or photographs, the tooth roots could not have been studied, 2) landmarks on small structures like the TMJ are easier and more precisely digitized on 3D-reconstructed skulls through zooming tools. Moreover, corrections are possible even in later stages of the analysis, and 3) lots of information is lost when landmarks are digitized on 2D images of complex three-dimensional structures (Cardini, 2014). In sum, although CT scanning and later 3D reconstruction is cost and time intensive, which reduces the achievable sample size, it was the only possible way to perform the analyses presented here.

The process of landmark digitization introduces some error in studies of geometric morphometrics. In order to rule out possible interobserver errors, the landmarks were only

digitized by one observer. In order to estimate the impact of the observer's error, a Procrustes ANOVA was performed in both studies (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002). The results showed that since the skull shape disparity in dogs is enormous, the error arising from inaccuracies in landmark placement is negligible, especially since only the first and highly significant partial least squares and principal components have been evaluated.

Another possible source of error is the ratio of landmarks to sample size. In studies of geometric morphometric, sample size should not exceed landmark number to produce valid results (Cardini and Elton, 2007; Cardini, 2014; Cardini et al., 2015b). Thus, in order to improve this ratio, landmarks were only digitized for one half of each skull. As Cardini (2016) showed, working with one half of a bilateral structure poses no problem for the result of a study as long as the shape variation in the sample is larger than what is expected from asymmetry. Again, because of the enormous disparity of domestic dog skulls (Drake and Klingenberg, 2010), asymmetries should create no significant difference to the results presented here.

For the analyses modules in the canine skull had to be defined. There is an ongoing debate on how modules can be delineated and which methods are suited to identify and validate a morphological module (see e.g. Magwene, 2009; Mitteroecker and Bookstein, 2009; Garcia et al., 2015). In the case of the mandible and the cranium, this thesis relied on widely accepted module definitions of previous studies which suggested a basic modular structure of the cranium in rostrum and braincase, and of the mandible in ramus and corpus (Drake and Klingenberg, 2010; Meloro and Slater, 2012), although there are more complex modularity scenarios proposed by now for both structures (Cheverud, 1996; Goswami and Polly, 2010; Anderson et al., 2014; Parr et al., 2016). The dentition was tested as a module apart from the rest of the skull in an exploratory way since previous studies suggested the partly independent inheritance and development of dental traits resulting in limited correlation of dentition and skull (Morey, 1992; Dayan et al., 2002; Cobourne and Sharpe, 2003; Boughner and Hallgrímsson, 2008; Gómez-Robles and Polly, 2012; Le Cabec et al., 2012; Asahara, 2013). Similarly, in the case of the TMJ, localized malformations suggested quasi-independence from the rest of the skull (Barrette, 1986; Vollmerhaus and Roos, 1996; Dickie et al., 2002).

The landmark sets were processed and analysed in MorphoJ (Klingenberg, 2011), PAST (Hammer et al., 2001) and the IMP series by H. David Sheets (Dept. of Physics, Canisius College, Buffalo, NY,USA). In these programs, analyses which are well established and

widely used in geometric morphometrics were used to approach the research questions in both studies. Principal component analyses (PCA) were used to analyse the principal axes of variation (Zelditch et al., 2012) and disparity values were calculated as a measure for the diversity of shapes (Foote, 1993). In partial least squares analyses (PLS) integration patterns were analysed (Rohlf and Corti, 2000; Klingenberg, 2013) and PLS vector angles and their significance were calculated to reveal differences in the integration pattern (Klingenberg and Marugan-Lobon, 2013). As a measure for integration strength, RV coefficients were calculated. This measure has been criticized in the past, especially since it varies with sample size and can thus pose a problem when groups of different sizes are compared (Fruciano et al., 2013). This criticism was considered by calculating a p-value in the RV comparison tool (Fruciano et al., 2013) that informs about the significance of RV coefficient differences.

As visualizations for the shape variation, both wire frame graphs and 3D-surface morphings were produced and deformed via “thin-plate-spline”-interpolation (Bookstein, 1989) using MorphoJ (Klingenberg, 2011) and “Landmark” (IDAV, University of California, Davis, USA). While wireframes are often less intuitive for interpretation, they are more accurate in their representation of landmarks and deformations. They were preferred in the first study because the shape variation was sufficiently represented by them. 3D surfaces can be interpreted more intuitively but show deformations in areas which were not digitized with landmarks and can thus be misleading. They were chosen for the second study since the complex shape deformations of the TMJ could not have been interpreted using wire frames only.

4 Conclusion and Outlook

The domestication history of modern dog breeds demonstrates on an intraspecific level the impressive radiation of skull shapes initiated by relaxed natural selective pressures and reinforced by intense selective breeding. Similar radiative processes happened frequently during organismal evolution resulting from population bottlenecks, through founder effects on island populations or after extinction events (e.g. Starck, 1953; Ruta et al., 2013; Blackburn et al., 2013; Magalhaes et al., 2016). Thus, the results presented in this thesis clearly reach beyond the species *Canis lupus* and will help to understand general patterns of diversification of skull shapes in nature. The central question of this thesis was whether a low integration and parcellation of the skull is required to generate new skull shapes and achieve high disparity levels. As it was shown, a large part of the disparity of skull shapes can arise from integrated variation along highly conserved covariation trajectories among skull modules, a scenario that is also likely in the early stages of natural radiation events. Even unusual and disproportional skull morphologies can be traced back to highly conserved patterns and are not a product of human intervention. Humans only picked spontaneously occurring forms such as mild brachycephalics and, by selective breeding, fostered the increasing severity of such traits. The skull, the teeth, the TMJ and the internal modules of the cranium and the mandible in domestic dogs and wolves covary mostly in predictable ways. Only on lower hierarchical levels, as in the case of the canine TMJ, the impact of localized factors can become more important for generating new shapes in single modules.

Studies in the future should try to replicate the results gained in this thesis for the skulls of wild carnivores to better understand natural radiation events of highly integrated structures. Especially covariation patterns of the tooth rows, the skull and the TMJ are not well studied and further investigations could help to understand their functional integration. Also for the domestic dog in particular, studying the process of mastication for differently shaped heads could prove informative, especially when combined with studies on the covariation of maloccluding teeth and TMJ shapes, since malocclusions might induce jaw movements that ultimately alter the shape of the TMJ.

5 SUMMARY

This thesis explores the concept of modularity and integration in the mammalian skull using domestic dogs and wolves as model organisms. Domestic dogs stand out due to an enormous morphological variation of their skulls, which clearly surpasses the variation found in their wild ancestors, the wolves (*Canis lupus*, Linnaeus 1758). A frequent phenomenon among this variation is the occurrence of disproportions between subunits, or modules, of the skull (such as the cranium and the mandible in bulldogs or the teeth and the mandible in small skulled dogs) which appear much more frequent and more pronounced in domestic dogs than in wolves. This phenomenon led up to the hypothesis that the integration of the skull modules in domestic dogs is degraded when compared to the wolf, so that the modules are structurally less dependent on each other than in the wolf. Moreover, it was hypothesized that the patterns of covariation of skull modules were altered through artificial selection. In a first study, this hypothesis was tested using methods from the field of geometric morphometrics on 196 CT scanned dog and wolf skulls. Other than expected, the results point to an unchanged integration strength of the skull modules in domestic dogs. Also the covariation pattern of skull modules is very similar to that of wolves. Through this surprising result, the hypothesis for a second study was formed. The temporomandibular joint of dogs is highly diverse when compared to the joint of wolves. The reasons for this phenomenon have not been explored before. If the high integration in the domestic dog skull does also apply to the temporomandibular joint, the shape of the joint should be predictable on the basis of skull geometry. Also this hypothesis was tested using geometric morphometrics with a sample of 274 CT scanned skulls. The results showed that certain characteristics of the temporomandibular joint can be traced back to overall skull geometry (foremost relative muzzle length, skull flexion and size). Exceptions from this pattern are frequent though, and dogs with very similar skulls can have differently formed jaw joints. This points to a certain degree of structural independence of the jaw joint from the rest of the skull. The observed covariation patterns in both studies closely resemble patterns found in primates and humans, other carnivores and even pigeons, which suggests a highly conserved developmental program that even in the case of the domestic dog could not be altered by human intervention.

6 ZUSAMMENFASSUNG

Diese Doktorarbeit beschäftigt sich mit der Modularität und Integration des Säugerschädels am Beispiel von Hunden und Wölfen. Hunde zeichnen sich durch eine sehr große morphologische Variation ihrer Schädel aus, die die Variation ihrer wilden Vorfahren, den Wölfen (*Canis lupus*, Linnaeus 1758), bei weitem übersteigt. Ein häufig zu beobachtendes Phänomen innerhalb dieser Variation ist das Auftreten von Mißverhältnissen zwischen einzelnen Schädelanteilen oder Modulen (z.B. von Unterkiefer und Cranium bei Bulldoggen oder dem Unterkiefer und den Zähnen bei kleinschädeligen Hunden), die bei Hunden deutlich häufiger und ausgeprägter auftreten als bei Wölfen. Dieses Phänomen führte zur Hypothese, dass die Integration einzelner Schädelmodule bei Hunden verglichen mit dem Wolf herabgesetzt ist, dass sich Module beim Hund also unabhängiger voneinander entwickeln, als beim Wolf. Auch wurde die Hypothese aufgestellt, dass die Muster der Kovariation dieser Schädelmodule beim Hund gegenüber dem Wolf durch künstliche Selektion verändert wurden. In einer ersten Studie wurde diese Hypothese mit den Methoden der geometrischen Morphometrie an 196 CT-Scan basierten 3D-Rekonstruktionen von Schädeln überprüft. Die Ergebnisse deuten entgegen der Erwartung nicht auf eine herabgesetzte Integration der Schädelmodule bei Hunden hin. Auch die Kovariationsmuster zwischen einzelnen Schädelmodulen sind denen von Wölfen sehr ähnlich. Durch dieses überraschende Ergebnis wurde die Hypothese für eine zweite Studie geprägt. Die Kiefergelenke von Hunden sind verglichen mit denen von Wölfen überaus divers. Die Gründe hierfür wurden bislang kaum erforscht. Sollte eine hohe Integration im Schädel auch für das Kiefergelenk von Hunden gelten, so sollte sich die Gestalt des Kiefergelenks auf die Schädelgestalt zurückführen lassen. Diese Hypothese wurde ebenso mit den Methoden der geometrischen Morphometrie an 274 CT-Scan basierten 3D-Rekonstruktionen von Schädeln getestet mit dem Ergebnis, dass sich gewisse Eigenschaften des Kiefergelenks tatsächlich durch die Morphologie des gesamten Schädels (vor allem relative Schnauzenlänge, Schädelknickung und -größe) erklären lassen. Ausnahmen von dieser Regel sind jedoch vielfältig und Hunde mit sehr ähnlichen Schädeln können durchaus sehr unterschiedliche Kiefergelenke aufweisen, was für einen gewissen Grad an struktureller Unabhängigkeit des Kiefergelenks vom Rest des Schädels spricht. Die beobachteten Muster der Kovariation der in beiden Studien untersuchten Schädelmodule gleichen den Mustern, die für Primaten, Menschen, andere Carnivoren und sogar Tauben beobachtet wurden und weisen auf hochgradig konservierte Entwicklungsprogramme hin, an denen beim Hund auch menschliche Intervention nichts ändern konnte.

7 REFERENCES

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APPENDIX

APPENDIX TO STUDY I

Supplementary material 1 (S1): Table 1 Sample composition.

FCI Group	Breed or Type	Specimens (n = 196)	Source/Collection
1 Sheep Dogs and Cattle Dogs	Australian Shepherd	2	VETSUI
	Bearded Collie	1	NMBE
	Berger de Beauce	1	NMBE
	Berger de Blanc Suisse	3	NMBE (1) VETSUI (2)
	Berger de Brie	1	NMBE
	Berger de Picardie	1	VETSUI
	Bobtail	1	NMBE
	Bouvier de Flandres	1	NMBE
	Collie	1	NMBE
	Dutch Shepherd	1	NMBE
	German Shepherd	5	VETSUI (3) PHYM (1) (NMBE (1)
	Groenendael	1	NMBE
	Komondor	1	NMBE
	Kuvasz	1	NMBE
	Sheltie	1	NMBE
	Slovensky Tschuwatsch	1	NMBE
	Tervueren	1	NMBE
	Welsh Corgi	1	NMBE
2 Pinscher, Schnauzer, Molossoids and Swiss Mountain Dogs	Appenzeller Mountain Dog	1	NMBE
	Bernese Mountain Dog	5	VETSUI (4) NMBE (1)
	Boxer	2	VETSUI (1) NMBE (1)
	Bullmastiff	1	VETSUI
	Doberman	1	NMBE
	English Bulldog	1	PHYM
	Entlebucher Mountain Dog	1	NMBE
	Great Dane	2	VESUI (1) NMBE (1)
	Great Swiss Mountain Dog	1	NMBE
	Hovawart	1	NMBE
	Landseer	1	NMBE
	Mastiff	1	NMBE
	Standard Schnauzer	2	PHYM (1) NMBE (1)
	Montagne des Pyrénées	1	NMBE
	Newfoundland	1	NMBE
	Pinscher	1	NMBE
	Giant Schnauzer	1	NMBE
	Rottweiler	3	VETSUI (2) PHYM (1)
	Shar Pei	1	NMBE
	Tibetan Mastiff (Do-Khyi)	1	NMBE
	Miniature Schnauzer	1	NMBE
3 Terrier	Airedale Terrier	1	NMBE
	Australian Terrier	1	NMBE
	Bullterrier	2	NMBE
	Foxterrier	1	NMBE
	Irish Terrier	1	NMBE

	German Hunting Terrier	1	NMBE
	Kerry Blue Terrier	1	NMBE
	Scottish Terrier	2	VETSUI (1) NMBE (1)
	Sealyham Terrier	1	NMBE
4 Dachshunds	Dachshund	2	VETSUI (1) NMBE (1)
5 Spitz and Primitive Types	Akita Inu	1	NMBE
	Alaskan Malamute	1	NMBE
	Basenji	1	VETSUI
	Canaan Dog	1	NMBE
	Chow Chow	2	VETSUI (1) NMBE (1)
	Finnish Spitz	1	NMBE
	Husky	3	VETSUI (1) NMBE (2)
	Greenland Dog	1	NMBE
	Pharao Dig	1	NMBE
	Samoyed	1	NMBE
	Spitz	1	NMBE
	Wolfspitz	2	LEIPZ (1) NMBE (1)
6 Scent Hounds	Basset Hound	1	VETSUI
	Beagle	2	VETSUI (1) NMBE (1)
	Bernese Scent Hound	1	NMBE
	Bloodhound	1	NMBE
	Briquet Griffon Vendeen	1	VETSUI
	Dalmatine	2	VETSUI (1) NMBE (1)
	Jura Hound	1	NMBE
	Austrian Bracke	1	NMBE
	Rhodesian Ridgeback	2	NMBE (1) PHYM (1)
7 Pointers and Setters	Gordon Setter	1	VETSUI
	Irish Setter	1	NMBE
	Small Münsterländer	2	VETSUI (1) NMBE(1)
	Pointer	1	NMBE
	German Pointer	3	NMBE
	Weimaraner	2	VETSUI (1) NMBE (1)
8 Retrievers, Flushing and Water Dogs	Chesapeake Bay Retriever	1	NMBE
	Cocker Spaniel	1	NMBE
	German Quail Dog	1	NMBE
	English Springer Spaniel	2	VETSUI
	Golden Retriever	5	VETSUI (3) PHYM (1)
	Labrador Retriever	3	VETSUI (1) PHYM (1) NMBE (1)
9 Companion	French Bulldog	1	NMBE
	Griffon Bruxellois	1	NMBE
	King Charles Spaniel	1	VETSUI
	King Charles Spaniel. Cavalier	1	NMBE
	Poodle (middle sized breed)	1	NMBE
	Poodle (large breed)	1	NMBE
	Poodle (small breed)	1	NMBE
10 Sight Hounds	Barsoi	1	NMBE
	Greyhound	2	PHYM (1) NMBE(1)
	Irish Wolfhound	1	NMBE

	Magyar agar	1	NMBE
	Saluki	1	VETSUI
	Scottish Deerhound	1	NMBE
	Sloughi	1	NMBE
	Whippet	1	NMBE
0 No Classification	Dingo	1	NMBE
	Haho-Awu	1	NMBE
	Pariah dog, Egypt	1	NMBE
	"Zughund"	1	NMBE
	Mixed Breed	1	VETSUI
	Wolf-Dog-Hybrid	1	SENCK
Wolves	*Canis lupus*	55	SENCK (9) DIGIM (1) NMBE (13) NMB (27) SMNS (3) PHYM (2)

NMBE	Natural History Museum, Berne (CH)
VETSUI	Vetsuisse Faculty, Berne (CH)
SENCK	Senckenbergmuseum of Natural History, Görlitz (GER)
PHYM	Phyletic Museum, Jena (GER)
DIGIM	digimorph.org, University of Texas
LEIPZ	Veterinary Faculty, Leipzig (GER)
NMB	Natural History Musem, Berlin (GER)
SMNS	State Museum of Natural History, Stuttgart (GER)

Supplementary material 2 (S2): Table 2 CT-scan specifications.

Place of Scanning	CT-Scanner	Scan	kvp (W)	Matrix (pixels)	Voxel Size (mm)	Filter
Vetsuisse Faculty, Berne (CH)	Philips Brilliance 16P	spiral	120	1024 x 1024	0.1 x 0.1 x 0.4	
Vetsuisse Faculty, Berne (CH)	CT-scanner: Philips Brilliance 16P	spiral	120	various formats		
Max-Planck-Institute for Evolutionary Anthropology, Leipzig (GER)	BIR ACTIS 225/300 high resolution industrial scanner	axial	130	2048 x 2048	0.1 x 0.1 x 0.1	0.50mm Brass
University Hospital, Jena (GER)	Medical Systems LightSpeed VCT	spiral	120	512 x 512	0.3 x 0.3 x 0.6	medium filter
University Hospital, Jena (GER)	Medical Systems LightSpeed VCT	spiral	120	512 x 512	0.3 x 0.2 x 0.6	medium filter
digimorph.org, University of Texas	High-Resolution X-ray CT Facility					
Veterinary Faculty, Leipzig (GER)	Philips Brilliance6	spiral	120	512 x 512	0.3 x 0.3 x 0.6	D
University Hospital, Jena (GER)	Medical Systems LightSpeed VCT	spiral	120	512 x 512	0.3 x 0.3 x 0.4	medium filter
University Hospital, Jena (GER)	Medical Systems LightSpeed VCT	spiral	120	512 x 512	0.3 x 0.3 x 0.4	medium filter

Supplementary Material 3 (S3): Table 3 Landmark definitions.

#	Group	Module	Description
1	Cranium	Muzzle	I1 left and right - Central point
2	Cranium	Muzzle	Os nasale - Anterior inner sutura
3	Cranium	Muzzle	Foramen infraorbitale - upper end
4	Cranium	Braincase	Os frontale - Processus zygomaticus
5	Cranium	Braincase	M. temporalis boundary - Half distance between LM 4 - LM7
6	Cranium	Braincase	Cranial vault - Medial plane from LM 5
7	Cranium	Braincase	Os interparietale - Most posterior point
8	Cranium	Braincase	Meatus acusticus externa
9	Cranium	Braincase	Foramen magnum - dorsal
10	Cranium	Braincase	Foramen magnum - ventral
11	Cranium	Braincase	Paraoccipital process
12	Cranium	Braincase	Proc. postglenoidalis
13	Cranium	Braincase	Os zygomaticus - Sutura squamosal-jugal - posterior end
14	Cranium	Braincase	Os temporalis - Sutura squamosal-jugal - anterior end
15	Cranium	Braincase, Muzzle	Zygomatic arch - Most anterior point on ventral surface
16	Cranium	Braincase, Muzzle	Orbita - Most anterior point
17	Cranium	Muzzle	C1 alveole posterior
18	Cranium	Muzzle	Incisive foramen - Posterior end
19	Cranium	Muzzle	P4 alveole posterior
20	Cranium	Braincase, Muzzle	Palatine - Most posterior point
21	Cranium	Braincase	Processus pterygoideus
22	Cranium	Braincase	Occipital condyle - Most central point
23	Cranium	Braincase	Occipital condyle - Most lateral point
24	Cranium	Braincase	Orbital fissure
25	Cranium	Braincase	Cranium medial plane between Proc. zygomaticus
26	Cranium	Braincase, Muzzle	Cranium medial plane between frontal orbita

#	Group		Description
27	Mandible	Corpus	Symphysis anterior end
28	Mandible	Corpus	Symphysis posterior end
29	Mandible	Corpus	C1 alveole posterior
30	Mandible	Corpus	P4 alveole posterior
31	Mandible	Corpus, Ramus	M3 alveole posterior
32	Mandible	Ramus	Processus condylaris medial
33	Mandible	Ramus	Processus condylaris lateral
34	Mandible	Ramus	Lowest point between coronoid and conylar process
35	Mandible	Ramus	Processus coronoideus - top
36	Mandible	Ramus	Processus angularis - Upper tip
37	Mandible	Corpus, Ramus	Frontal end Fossa masseterica
38	Mandible	Corpus	Ramus mandibula most ventral point
39	Mandible	Corpus	Foramen mentalia anterior
#	Group		Description
40	Upper Dentition		I1 tip
41	Upper Dentition		I1 tip of root
42	Upper Dentition		I2 tip
43	Upper Dentition		I2 tip of root
44	Upper Dentition		I3 tip
45	Upper Dentition		I3 tip of root
46	Upper Dentition		C1 tip
47	Upper Dentition		C1 tip of root
48	Upper Dentition		P1 paracone tip
49	Upper Dentition		P1 tip of root
50	Upper Dentition		P2 paracone tip
51	Upper Dentition		P2 tip of root mesial
52	Upper Dentition		P2 tip of root distal
53	Upper Dentition		P3 paracone tip

54	Upper Dentition		P3 tip of root mesial
55	Upper Dentition		P3 tip of root distal
56	Upper Dentition		P4 paracone tip 1
57	Upper Dentition		p4 metastyl tip 2
58	Upper Dentition		P4 protocone
59	Upper Dentition		P4 root tip mesial
60	Upper Dentition		P4 root tip distal
61	Upper Dentition		P4 root tip lingual
62	Upper Dentition		M1 tip paracone
63	Upper Dentition		M1 tip metacone
64	Upper Dentition		M1 lingualmost point
65	Upper Dentition		M1 roottip mesial
66	Upper Dentition		M1 roottip distal
67	Upper Dentition		M1 rootip lingual
68	Upper Dentition		M2 central fossa
69	Upper Dentition		M2 root tip mesial
70	Upper Dentition		M2 root tip distal
#	**Group**		**Description**
71	Lower Dentition		I1 tip
72	Lower Dentition		I1 root tip
73	Lower Dentition		I2 tip
74	Lower Dentition		I2 root tip
75	Lower Dentition		I3 tip
76	Lower Dentition		I3 root tip
77	Lower Dentition		C1 tip
78	Lower Dentition		C1 root tip
79	Lower Dentition		P1 paracone tip
80	Lower Dentition		P1 root tip
81	Lower Dentition		P2 paracone tip

82	Lower Dentition		P2 root tip mesial
83	Lower Dentition		P2 root tip distal
84	Lower Dentition		P3 paracone tip
85	Lower Dentition		P3 root tip mesial
86	Lower Dentition		P3 root tip distal
87	Lower Dentition		P4 paracone tip
88	Lower Dentition		P4 root tip mesial
89	Lower Dentition		P4 root tip distal
90	Lower Dentition		M1 protocone tip
91	Lower Dentition		M1 paracone tip
92	Lower Dentition		M1 metacone tip
93	Lower Dentition		M1 mesial root tip
94	Lower Dentition		M1 distal root tip
95	Lower Dentition		M2 hypocone tip
96	Lower Dentition		M2 mesial root tip
97	Lower Dentition		M2 distal root tip
98	Lower Dentition		M3 tip
99	Lower Dentition		M3 root tip

Supplementary Material 4 (S4): Table 4 Results of the Procrustes ANOVA.

Centroid size					
Effect	SS	MS	df	F	P (param.)
Individual	281816.36	31312.93	9	20769.29	<0.0001
Intraobserver Error	30.153107	1.51	20		
Shape					
Effect	SS	MS	df	F	P (param.)
Individual	0.3317106	0.00012709	2610	81.48	<0.0001
Intraobserver Error	0.0090469	0.00000156	5800		

Supplementary Material 5.1 (S5.1): Allometry of the cranium and the mandible (including dentition) in wolves and dogs.

The effect of differing centroid sizes on the shape of the cranium and the mandible was analysed separately for both wolves and dogs via discriminant function and subsequent calculation of vector angles between wolves and dogs.

The allometric effect is very similar as reflected by significant vector angles (cranium: 54.4°, $p < 0.00001$; mandible: 49.95°, $p < 0.00001$). Also the shapes associated with small and large specimens are very similar (Fig. 1). The mandibles of large specimens are robust, with a large dorso-ventral diameter and large muscle attachment sites on the ramus. The teeth are relatively small compared to the size of the mandible. Small specimens have gracile mandibles with a small ascending ramus, small dorso-ventral diameter of the corpus and relatively large teeth. The cranium of large specimens is robust with a high and voluminous muzzle, a sagittal crest on the braincase, relatively small orbits and relatively small teeth. The cranium of small specimens has a rather globular braincase, a small muzzle, relatively large orbits and large teeth.

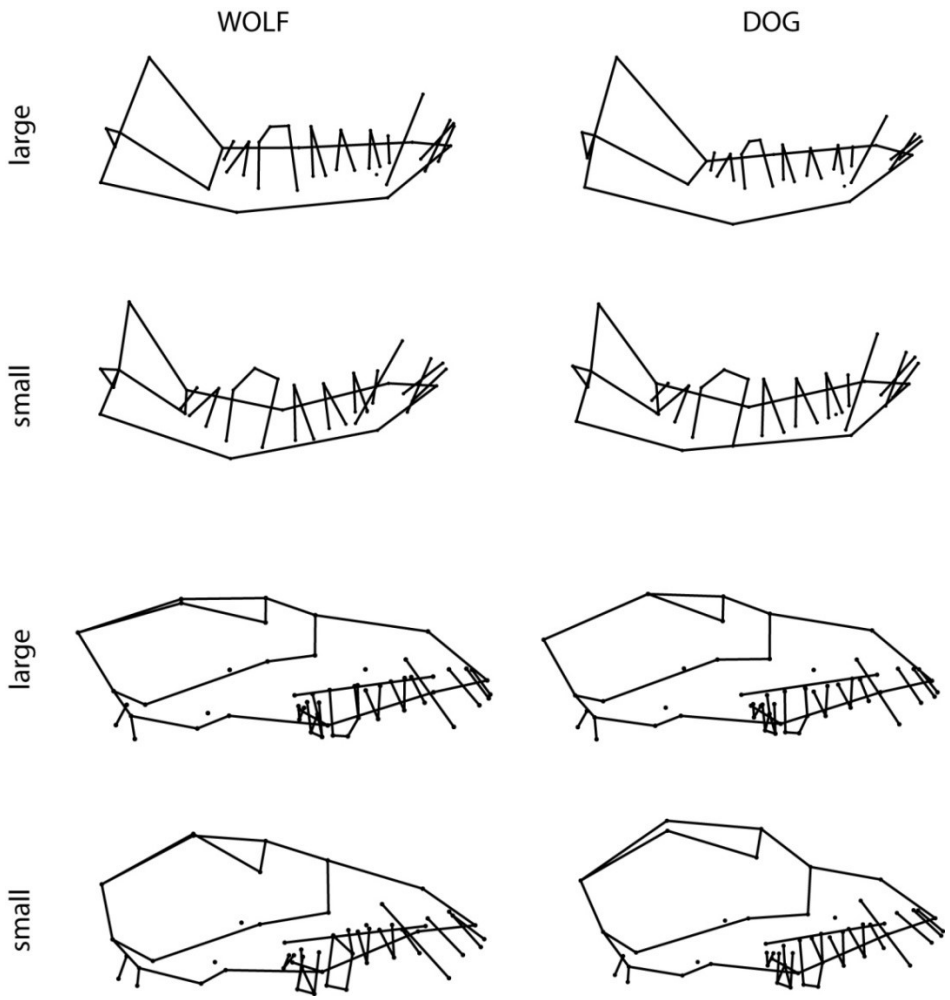

Fig. 1 Allometric effect on cranium and mandible in wolves and dogs.

Supplementary Material 5. 2 (S5.2): Correlation of centroid size and PLS scores

The tests for a correlation of centroid size and PLS scores of the PLS analyses performed in our study gave no significant results, apart from two exceptions (Table 1). The exceptions were in Block 1 (braincase) in PLS 2 and 3 of the PLS testing the covariation of muzzle and braincase in the cranium of wolves. These two PLS axes were significantly correlated to size changes (PLS 2: r = 0.43; p = 0.001; PLS 3: r = -0.27; p = 0.0477). With these results we would evaluate allometric influences as not threatening for the results of our study. The cranial and mandibular allometry in wolves and dogs is very similar and the influence on the PLS analyses is minimal and mostly not significant.

Table 1. Pearson correlation tests of centroid size and PLS scores

CRANIUM vs. UPPER TEETH	**Dog**	**Dog**	**Wolf**	**Wolf**		**Dog**	**Dog**	**Wolf**	**Wolf**
	r	**p**	**r**	**p**		**r**	**p**	**r**	**p**
Block 1 PLS1	-0.01	0.8875	0.19	0.1661	Block 2 PLS1	0.11	0.1908	-0.19	0.1580
Block 1 PLS2	-0.08	0.3325	0.11	0.3989	Block 2 PLS2	-0.02	0.8234	0.08	0.5785
Block 1 PLS3	-0.11	0.2150	0.05	0.7329	Block 2 PLS3	-0.01	0.8762	-0.07	0.6187
Block 1 PLS4	0.10	0.2596	-0.15	0.2720	Block 2 PLS4	0.03	0.7218	0.07	0.6340
MANDIBLE vs. LOWER TEETH	**Dog**	**Dog**	**Wolf**	**Wolf**		**Dog**	**Dog**	**Wolf**	**Wolf**
	r	**p**	**r**	**p**		**r**	**p**	**r**	**p**
Block 1 PLS1	-0.09	0.3014	0.05	0.7272	Block 2 PLS1	0.02	0.8173	-0.08	0.5438
Block 1 PLS2	0.01	0.8695	-0.24	0.0770	Block 2 PLS2	0.00	0.9954	-0.13	0.3508
Block 1 PLS3	0.02	0.8060	0.11	0.4380	Block 2 PLS3	0.06	0.5123	-0.01	0.9657
Block 1 PLS4	0.04	0.6770	-0.05	0.7171	Block 2 PLS4	-0.04	0.6003	0.00	0.9729
MUZZLE vs. BRAINCASE	**Dog**	**Dog**	**Wolf**	**Wolf**		**Dog**	**Dog**	**Wolf**	**Wolf**
	r	**p**	**r**	**p**		**r**	**p**	**r**	**p**
Block 1 PLS1	0.09	0.2679	0.08	0.5736	Block 2 PLS1	-0.03	0.7304	0.21	0.1242
Block 1 PLS2	0.05	0.5373	**0.43**	**0.0010**	Block 2 PLS2	0.02	0.7742	0.04	0.7977
Block 1 PLS3	0.03	0.7583	**-0.27**	**0.0477**	Block 2 PLS3	0.06	0.4680	-0.07	0.6159
Block 1 PLS4	0.06	0.4490	0.15	0.2663	Block 2 PLS4	0.09	0.2661	0.13	0.3345
RAMUS vs. CORPUS	**Dog**	**Dog**	**Wolf**	**Wolf**		**Dog**	**Dog**	**Wolf**	**Wolf**
	r	**p**	**r**	**p**		**r**	**p**	**r**	**p**
Block 1 PLS1	0.02	0.8523	0.02	0.8949	Block 2 PLS1	-0.08	0.3334	-0.13	0.3518
Block 1 PLS2	0.03	0.7677	-0.12	0.3844	Block 2 PLS2	-0.07	0.4188	-0.15	0.2625
Block 1 PLS3	0.03	0.7300	0.07	0.5843	Block 2 PLS3	-0.04	0.6659	0.02	0.8934
Block 1 PLS4	0.02	0.8459	-0.11	0.4223	Block 2 PLS4	0.01	0.8749	0.16	0.2279

Supplementary Material 6 (S6): Sexual dimorphism in wolves and dogs.

We tested for differences concerning the sexual dimorphism in wolves and dogs by performing a discriminant function on the shape data of mandible and cranium (including teeth). The tests revealed very similar shapes associated with both sexes in both groups. Males have mandibles with relatively large dorso-ventral corpus diameter and a large ascending ramus. The teeth are small relative to the size of the mandible (the effect here is stronger in dogs). Female mandibles are slender with a small ascending ramus and hence relatively large teeth. Male crania have a voluminous muzzle, small orbits and a braincase which is posteriorly extended by occipital and sagittal crests. Female crania lack this posterior extension and tend towards a roundish outline of the braincase. The orbits are relatively large and the muzzle anterior-posteriorly long and rather slender. In dogs, females tend to a more pronounced forehead. Since in both groups a random mixture of males and females was included and both dogs and wolves show similar signs of sexual dimorphism, we evaluate this factor as negligible for the given study.

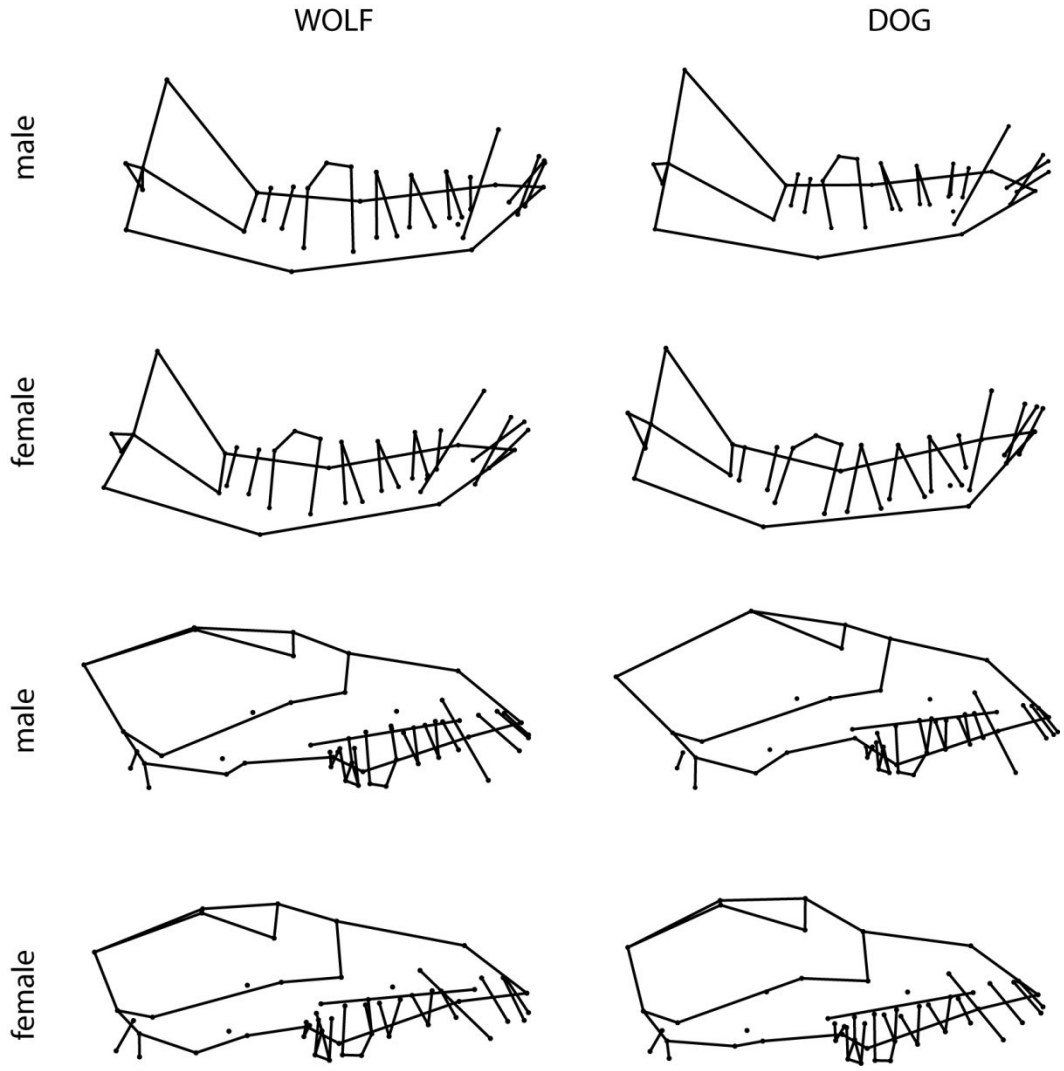

Fig. 1 Sexual dimorphism in wolves and dogs (based on a discriminant function, performed separately for both groups).

Supplementary Material 7 (S7): Table 1 Module variance in dogs and wolves as explained by the covariation with other modules.

Module	in covariation with…		% of variation (Dogs)	% of variation (Wolves)
Braincase	Rostrum	PLS 1	23.9	7.4
		PLS 2	7.5	4.9
		PLS 3	4.9	5.1
		PLS 4	4.1	2.6
Rostrum	Braincase	PLS 1	16.2	3.9
		PLS 2	2.0	4.7
		PLS 3	1.4	1.6
		PLS 4	0.7	2.8
Cranium	Upper Dentition	PLS 1	31.2	8.5
		PLS 2	6.1	7.3
		PLS 3	4.9	4.7
		PLS 4	1.8	3.4
Upper Dentition	Cranium	PLS 1	25.7	4.9
		PLS 2	4.0	5.0
		PLS 3	4.2	2.7
		PLS 4	1.7	2.0
Mandible	Lower Dentition	PLS 1	22.3	10.0
		PLS 2	9.2	6.7
		PLS 3	4.7	8.4
		PLS 4	3.5	2.9
Lower Dentition	Mandible	PLS 1	21.2	13.0
		PLS 2	8.8	5.4
		PLS 3	4.1	3.7
		PLS 4	4.1	4.3
Ramus	Corpus	PLS 1	24.8	3.1
		PLS 2	4.8	2.4
		PLS 3	3.9	4.2
		PLS 4	1.4	1.4
Corpus	Ramus	PLS 1	24.8	7.3
		PLS 2	4.1	5.9
		PLS 3	4.1	1.6
		PLS 4	1.7	2.2

APPENDIX TO STUDY II

Supplementary Material 1 (S1): Table 1 Sample composition.

Group (according to the Fédération Cynologique Internationale)	Breeds or Varieties (n = 129)	Specimens (n = 274)
1 Sheep Dogs and Cattle Dogs	Australian Shepherd	3
	Bearded Collie	1
	Berger de Beauce	1
	Berger de Blanc Suisse	3
	Berger de Brie	1
	Berger de Picardie	1
	Bobtail	1
	Bouvier de Flandres	1
	Collie	1
	Dutch Shepherd	1
	German Shepherd	5
	Groenendael	1
	Komondor	1
	Kuvasz	1
	Puli	1
	Sheltie	1
	Slovensky Tschuwatsch	1
	Tervueren	1
	Welsh Corgi	1
2 Pinscher, Schnauzer, Molossoids and Swiss Mountain Dogs (n = 42)	Appenzell Mountain Dog	1
	Bernese Mountain Dog	8
	Boxer	4
	Bullmastiff	2
	Doberman	1
	Dogue de Bordeaux	1
	English Bulldog	3
	Entlebuch Mountain Dog	1
	Giant Schnauzer	1
	Great Dane	1
	Great Swiss Mountain Dog	1
	Hovawart	1
	Landseer	1
	Leonberger	1
	Mastiff	1
	Mastino Napoletano	1
	Miniature Pinscher	1
	Montagne des Pyrénées	1
	Newfoundland	1

	Pinscher	1
	Rottweiler	4
	Shar Pei	1
	Standard Schnauzer	2
	Tibetan Mastiff (Do-Khyi)	1
	Miniature Schnauzer	1
3 Terriers (n = 21)	Airedale Terrier	1
	Australian Terrier	1
	Bedlington Terrier	1
	Black and Tan Terrier	1
	Border Terrier	1
	Bullterrier	2
	Foxterrier	1
	Irish Terrier	1
	Jack Russel Terrier	2
	German Hunting Terrier	1
	Kerry Blue Terrier	1
	Norfolk Terrier	1
	Norwich Terrier	2
	Scottish Terrier	2
	Sealyham Terrier	1
	Westhighland White Terrier	1
	Skye Terrer	1
4 Dachshunds (n = 5)	Dachshund	5
5 Spitz and Primitive Types (n = 21)	Akita Inu	1
	Alaskan Malamute	1
	Basenji	2
	Canaan Dog	1
	Chow Chow	2
	Finnish Spitz	1
	Husky	3
	Greenland Dog	1
	Iceland Dog	1
	Miniature Spitz	1
	Pharao Dog	1
	Samoyed	1
	Spitz	1
	Wolfspitz	3
	Xoloitzcuintli	1
	Beagle	2
	Basset Hound	1

6 Scent Hounds (n = 13)	Basset Griffon Vendeen	1
	Bernese Scent Hound	1
	Bloodhound	1
	Briquet Griffon Vendeen	1
	Dalmatine	2
	Jura Hound	1
	Austrian Bracke	1
	Rhodesian Ridgeback	2
7 Pointers and Setters (n = 10)	Gordon Setter	1
	Irish Setter	1
	Small Münsterländer	2
	Pointer	1
	German Pointer	3
	Weimaraner	2
8 Retrievers, Flushing and Water Dogs (n = 21)	Chesapeake Bay Retriever	1
	Cocker Spaniel	2
	German Quail Dog	1
	English Springer Spaniel	2
	Golden Retriever	8
	Labrador Retriever	7
9 Companion Dogs (n = 22)	Bichon Havanais	1
	Chihuahua	2
	Chinese Crested	1
	English Toy Terrier	1
	French Bulldog	5
	Griffon Bruxellois	1
	King Charles Spaniel	4
	Pekinese	1
	Poodle (middle sized breed)	2
	Poodle (large breed)	1
	Poodle (small breed)	1
	Pug	1
	Tibet Spaniel	1
10 Sight Hounds (n = 13)	Afghan	1
	Borzoi	1
	Greyhound	2
	Irish Wolfhound	1
	Italian Greyhound	1
	Magyar agar	1
	Saluki	1
	Scottish Deerhound	1
	Sloughi	1
	Whippet	3
	Dingo	1

No Classification (n = 20)	Haho-Awu	1
	Pariah dog, Egypt	1
	Rat Catcher	1
	"Zughund"	1
	Mixed Breed	14
	Wolf-Dog-Hybrid	1
Wolves (n = 60)	*Canis lupus*	60

Supplementary Material 2 (S2): Table 2 Museum collections and CT-scan specifications.

Museum collections and CT-scan sources	n
Natural History Museum, Berne (CH)	136
Vetsuisse Faculty, Berne (CH)	38
Senckenbergmuseum of Natural History, Görlitz (GER)	15
Phyletic Museum, Jena (GER)	9
digimorph.org, University of Texas	1
Veterinary Faculty, Leipzig (GER)	1
Natural History Musem, Berlin (GER)	27
State Museum of Natural History, Stuttgart (GER)	2

Scanning facilities	n	CT-Scanner	Scan	Matrix (pixels)	Slice Thickness (mm)
Vetsuisse Faculty, Berne (CH)	159	Philips Brilliance 16P	spiral	1024 x 1024	0.4
University Hospital, Jena (GER)	59	Medical Systems LightSpeed VCT	spiral	512 x 512	0.625
Max-Planck-Institute for Evolutionary Anthropology, Leipzig (GER)	5	BIR ACTIS 225/300	axial	2048 x 2048	0.091
Hannover Veterinary Faculty (GER)	1	Phillips Brilliance 64	spiral	512 x 512	0.625
Digimorph.org, University of Texas	1	High-Resolution X-ray CT Facility	spiral	513 x 442	0.5
Veterinary faculty, Leipzig (GER)	1	Philips Brilliance 6	spiral	512 x 512	0.313

Supplementary Material 3 (S3): Table 3 Landmark definitions.

#		Group	Description
1	1	Cranium	Os incisivum - mesial point between left and right I1
2	2	Cranium	Os nasale - Sutura internasalis, rostral end
3	3	Cranium	Os frontale - Processus zygomaticus
4	4	Cranium	Calvaria - Linea temporalis - half distance between LM 4 - LM7
5	5	Cranium	Calvaria - medial plane from LM 5
6	6	Cranium	Protuberantia occipitalis externa
7	7	Cranium	Meatus acusticus externus
8	8	Cranium	Foramen magnum - dorsal
9	9	Cranium	Foramen magnum - ventral
10	10	Cranium	Os zygomaticus - Sutura temporozygomatica - caudal end
11	11	Cranium	Os temporalis - Sutura temporozygomatica - rostral end
12	12	Cranium	Sutura zygomaticomaxillaris - ventral surface of Os zygomaticum
13	13	Cranium	Margo infraorbitalis - most rostral point
14	14	Cranium	Os maxillare - C1 alveole distal
15	15	Cranium	Os maxillare - P4 alveole distal
16	16	Cranium	Os palatinum - most caudo-medial point
17	17	Cranium	Calvaria - medial plane between Proc. zygomaticus (LM 3)
18	18	Cranium	Os nasale - medial plane between frontal orbita (LM 13)
#		**Group**	**Description**
19	1	Mandible	Mandibular symphysis rostral end
20	2	Mandible	Mandibular symphysis caudal end
21	3	Mandible	C1 alveole distal
22	4	Mandible	P4 alveole distal/M1 alveole mesial
23	5	Mandible	M3 alveole distal
24	6	Mandible	Depression between Proc. coronoideus and the condylar process
25	7	Mandible	Processus coronoideus - dorsal end
26	8	Mandible	Processus angularis - caudal tip
27	9	Mandible	Fossa masseterica - rostral end
28	10	Mandible	Ramus mandibulae most ventral point
29	11	Mandible	Foramen mandibulae anterior
#		**Group**	**Description**
30	1	Mandibular fossa	Processus retroglenoidalis medio-ventral end
31	2	Mandibular fossa	Processus retroglenoidalis most medial point
32	3	Mandibular fossa	Processus retroglenoidalis medial onset
33	4	Mandibular fossa	Most medial point joint surface
34	5	Mandibular fossa	Rostral midpoint joint surface
35	6	Mandibular fossa	Most lateral point joint surface
36	7	Mandibular fossa	Processus retroglenoidalis lateral onset
37	8	Mandibular fossa	Midpoint LM 1-7 on outline
38	9	Mandibular fossa	Midpoint LM 3-7 on joint surface

39	10	Mandibular fossa	Crossing of connection LM 4-6 9-5
40	11	Mandibular fossa	Midpoint LM 2-8
41	12	Mandibular fossa	Midpoint LM 5-7
#		**Group**	**Description**
42	1	Condylar process	Caput mandibulae - most lateral extension
43	2	Condylar process	Caput mandibulae - most medial extension
44	3	Condylar process	Caput mandibulae - rostro-lateral point on joint surface
45	4	Condylar process	Caput mandibulae - caudo-lateral point on joint surface
46	5	Condylar process	Caput mandibulae - caudo-lateral joint surface contour maximal medial bend
47	6	Condylar process	Caput mandibulae - rostro-medial point joint surface
48	7	Condylar process	Caput mandibulae - caudo-medial end joint surface
49	8	Condylar process	Caput mandibulae - caudal end of joint surface
50	9	Condylar process	Caput mandibulae - rostral end of joint surface
51	10	Condylar process	Caput mandibulae - midpoint LM 6-5 on joint surface
52	11	Condylar process	Caput mandibulae - midpoint LM 5-7 on joint surface
53	12	Condylar process	Caput mandibulae - midpoint LM 4-9 on joint surface

Supplementary Material 4 (S4): Table 4 The results of multiple Procrustes ANOVAs performed separately for the centroid size and shape of the cranium, the mandible, the mandibular fossa and the condylar process in order to determine the measurement error introduced by landmark placement.

CRANIUM								
Centroid Size								
Effect	SS	MS	df	F	p (param.)			MS %
Individuals	128985.22	9213.23	14	19382.28	<0.0001			99.9
Error Replicates	14.260288	0.475343	30					0.005
Shape								
Effect	SS	MS	df	F	p (param.)			MS %
Individuals	0.3563411	0.0005416	658	66	<0.0001			98.5
Error Replicates	0.0115691	8.205E-06	1410					1.4

MANDIBLE								
Centroid size								
Effect	SS	MS	df	F	p (param.)			MS %
Individuals	64340.571	4595.755	14	10232.88	<0.0001			99.9
Error Replicates	13.473501	0.449117	30					0.009
Shape								
Effect	SS	MS	df	F	p (param.)	Pillai tr.	P (param.)	MS %
Individuals	0.2244585	0.0006166	364	34.5	<0.0001	13.4	<.0001	97.1
Error Replicates	0.0139408	1.787E-05	780					2.8

MANDIBULAR FOSSA								
Centroid Size								
Effect	SS	MS	df	F	p (param.)			MS %
Individuals	1974.1138	141.00813	14	405.77	<0.0001			99.7
Error Replicates	10.425254	0.347508	30					0.2
Shape								
Effect	SS	MS	df	F	p (param.)	Pillai tr.	P (param.)	MS %
Individuals	0.7334903	0.0018066	406	14.41	<0.0001	12.6	<.0001	93.5
Error Replicates	0.1090919	0.0001254	870					6.4

CONDYLAR PROCESS								
Centroid size								
Effect	SS	MS	df	F	p (param.)			MS %
Individual	2004.0048	143.1432	14	970.88	<0.0001			99.8
Error	4.423085	0.147436	30					0.1

Shape								
Effect	SS	MS	df	F	P (param.)	Pillai tr.	P (param.)	MS %
Individual	0.739829	0.0018222	406	11.11	<0.0001	12.6	<.0001	91.7
Error	0.142632	0.0001639	870					8.2

SS	sums of squares
MS	mean squares
df	degrees of freedom
F	F-statistics (Goodall's)
P (param.)	p-value for significance
Pillai tr.	Pillai's trace
MS %	percentage of total mean squares

EHRENWÖRTLICHE ERKLÄRUNG

Hiermit erkläre ich, dass ich die vorliegende Dissertation „Modularity and Integration in the Skull of *Canis lupus* (Linnaeus 1758): A Geometric Morphometrics Study on Domestic Dogs and Wolves“ selbst angefertigt habe. Es wurden keine Textabschnitte eines Dritten ohne Kennzeichnung verwendet. Auch sind in diese Arbeit keine eigenen Arbeiten aus vorhergehenden Prüfungen eingeflossen. Alle von mir benutzten Hilfsmittel, persönliche Mitteilungen und Quellen sind in der Arbeit angegeben. Bei der Erstellung der in der vorliegenden Arbeit verwendeten Originalarbeiten haben Martin S. Fischer (FSU Jena) und Kornelius Kupczik (MPI Leipzig) als Autoren mitgewirkt. Mein Eigenanteil an den Publikationen ist jedoch jeweils angegeben (siehe Abschnitt 2 Published Results). Die Hilfe eines Promotionsberaters habe ich nicht in Anspruch genommen. Dritte haben weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Ich versichere weiterhin, dass ich die vorliegende Arbeit nicht für eine staatliche oder andere wissenschaftliche Prüfung als Prüfungsarbeit und auch an keiner anderen Hochschule als Dissertation eingereicht habe. Die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena ist mir bekannt.

Jena, 25.10.2017

Overview of Central Statements

Thesenübersicht

1) The skulls of domestic dogs are not less integrated than wolf skulls.
   Die Schädel von Hunden sind nicht schwächer integriert als die von Wölfen.

2) The skull modules of domestic dogs covary in ancestral (wolf-like) patterns.
   Die Schädelmodule von Hunden kovariieren nach wolfsähnlichen und damit ursprünglichen Mustern.

3) These covariation patterns reflect the main types of variation known for dog skulls which implies 'global' and shared and not 'local' and module-specific factors as the main driving forces for the diversification of *Canis lupus* skulls.
   Diese Kovariationsmuster spiegeln die Haupttypen der Schädelvariation bei Hunden wider, was impliziert, dass „globale" und geteilte und nicht „lokale", modulspezifische Faktoren die wichtigste treibende Kraft bei der Diversifikation des Schädels bei *Canis lupus* sind.

4) Although disproportions between the skull modules occur in domestic dogs, they occur in predictable ways and thus stand in no conflict with the high integration of the skull.
   Obwohl Mißverhältnisse zwischen den Schädelmodulen von Hunden auftreten, sind sie doch vorhersagbar und stehen daher nicht im Konflikt mit der hohen Integration des Schädels.

5) The temporomandibular joint (TMJ) displays a larger shape disparity in domestic dogs than in wolves.
   Das Kiefergelenk zeigt bei Hunden eine größere Gestaltsdisparität als bei Wölfen.

6) The TMJ of *Canis lupus* covaries with skull size, flexion and rostro-caudal skull extension, which is why certain features of it are predictable based on skull shape.
   Das Kiefergelenk von *Canis lupus* kovariiert mit der Größe des Schädels, der Schädelknickung und der rostro-caudalen Schädelausdehnung, was eine gewisse Vorhersagbarkeit von Gelenkeigenschaften auf Basis der Schädelgestalt möglich macht.

7) In spite of this predictability, relatively homogenous dog skulls can have differently shaped TMJs, showing that the integration of the joint in the dog skull is limited.
   Trotz dieser Vorhersagbarkeit können relativ homogene Schädel sehr verschieden geformte Gelenke aufweisen, was die begrenzte Integration des Gelenks im Schädel aufzeigt.

8) The diversification of the skull can and does happen under high integration in *Canis lupus* and other amniotes showing that large disparity can be achieved in spite of a low degree of modularity.
   Die Diversifikation des Schädels erfolgt unter hoher Integration bei *Canis lupus* und bei anderen Amnioten, was zeigt, dass große Gestaltsdisparität auch bei geringer Modularität erreicht werden kann.

9) The main patterns of integration and variation of the skull are shared by *Canis lupus* and other amniotes which implies the deep-time conservation of underlying factors.
   Die grundlegenden Muster der Integration und Variation des Schädels werden von *Canis lupus* und anderen Amnioten geteilt, was die Langzeit-Konservierung von Faktoren, die dieser Variation zugrunde liegen, impliziert.

10) In small skeletal structures like the TMJ, modularity might be increased due to local and dissociating factors such as force induced bone growth.
    In kleinen Skelettstrukturen wie dem Kiefergelenk könnte die Modularität durch lokale und dissoziierende Faktoren (wie etwa kraftinduziertem Knochenwachstum) eine größere Rolle spielen.